1976

Rapid Methods for the Bacteriological Analyses of Raw Frozen Breaded Shrimp.

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RAPID METHODS FOR THE BACTERIOLOGICAL ANALYSES OF RAW FROZEN BREADED SHRIMP

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Food Science

by
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B.S., Southeastern Louisiana University, 1970
M.S., Southeastern Louisiana University, 1972
May, 1976

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ACKNOWLEDGMENT

The author wishes to express sincere gratitude to his major professor, Dr. Robert M. Grodner, for his guidance and aid throughout this investigation.

He also wishes to express his thanks to Dr. Arthur F. Novak, Head of the Department of Food Science, Dr. Samuel P. Meyers, Dr. R. M. Rao, Dr. James E. Rutledge, and Dr. William F. McKnight for serving on his examination committee.

Gratitude is also expressed to Dr. William James of the Department of Food Science and Dr. Kenneth Koonce of the Department of Experimental Statistics for their assistance in the statistical analysis involved in the study.

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ABSTRACT

The raw frozen breaded shrimp industry is uniquely susceptible to bacteriological problems since there is no point in the processing which reduces the bacterial numbers appreciably. In addition, the time required to complete the processing of raw materials is less than 48 hours. The bacterial tests used to assess the quality of the finished product require up to 96 hours. Thus, such official techniques are of little use since the product may be past the retail outlet and in the hands of the consumer upon completion.

Rapid bacteriological procedures for the determination of aerobic plate count (APC), coliforms, E. coli, and coagulase-positive staphylococci were developed and compared to official methods on an interlaboratory basis. Eighteen replicates of identical samples of frozen breaded shrimp were analyzed by official methods and the rapid methods developed by the investigators. Each sample was sent to the LSU laboratory by one of 10 processors, homogenized, and identical homogenates sent to the 16 analytical laboratories. The analytical laboratories sent the data resulting from the coded samples back to the LSU investigators. All laboratories performed APC, coliform, E. coli, and coagulase-positive staphylococci analyses using both the official and

proposed rapid techniques.

The rapid APC procedure was a 24-hour plate count technique using Eugon agar. No significant difference ($p > 0.05$) was found between the techniques and the interaction, laboratory x technique was not significant ($p > 0.05$). No difference was noted between the analytical laboratories. The overall APC for the breaded shrimp ranged from 3.0×10^3/g to 5.5×10^6/g, with 25% of the samples having 1.0×10^6/g or more.

No significant difference ($p > 0.05$) was found between the 24-hour LST MPN for total coliforms and the official 4-day technique. The bivariate interaction, technique x laboratory, was not significant ($p > 0.05$). Broad variation in coliform MPN/g of shrimp was noted for the source and a significant difference ($p < 0.05$) found between the analytical laboratories. The overall range reported for coliforms in frozen breaded shrimp was 23/g to 1100/g with a mean of 185.9/g. Eight percent of the samples contained 1.0×10^3 coliforms/g or more.

A significant difference ($p < 0.05$) was not noted between techniques for E. coli. The rapid procedure entailed a 3 x 3 MPN in LST for 24 hours at 44.5°C. The interaction, laboratory x technique was not significant ($p > 0.05$). The overall range for E. coli was 0 to 60/g, with an overall mean of 2.5/g. Sixteen percent of the samples were positive for E. coli.

The rapid procedure for coagulase-positive

staphylococci was a 24-hour MPN procedure in TMM broth at 37°C. A significant difference ($p < 0.05$) was noted between the methods and analytical laboratories. A broad variation was found between the sources of the breaded shrimp samples. The overall range for coagulase-positive staphylococci using the official method was 0 to 1100/g. Fifty-nine percent of the samples were reported to contain coagulase-positive staphylococci, with an overall of only 10% of the samples having 100/g or more.

The rapid procedures tested are a reliable source of relatively rapid data for the frozen food industry. The bacteriological profile of the product is known before the product is distributed to the retail outlets, and if used continuously, abnormal bacterial numbers during processing are detected 72 hours earlier than that using the official techniques.

INTRODUCTION

The raw frozen seafood industry is uniquely susceptible to bacteriological problems. Their products undergo no bacteriocidal procedure other than the slow dying off associated with the frozen state. Consequently, the bacteriological analysis of the raw product, in-line monitoring, and finished product are of utmost importance. Unfortunately, bacteriological tests using the official methods require up to 4 days, the maximum duration allowed by the U.S. Food and Drug Administration (FDA) for data compilation. In that time the frozen breaded product has been distributed, placed on retail shelves, and could easily be at the consumer's table. More rapid tests are needed to allow the processor to know the bacterial quality of his product from raw material to finished product.

Rapid methods for the quality tests, aerobic plate count (APC), E. coli, coliforms, and coagulase-positive staphylococci have been developed. The time required for these analyses have varied, however. E. coli and coliform procedures using labeled lactose require as little as one hour (Levine et al., 1957). Rapid methods for APC requiring 4 hours have been reported by Winter et al. (1971) using microcolony counting on membrane filters. These procedures

are expensive, however, and require expertise not found in the typical industrial laboratory. An inexpensive, 24-hour technique has been described in official publications (U.S. Dept. of Health, Education, and Welfare, 1968) for coagulase-positive staphylococci. The procedure, however, is not recommended for samples containing greater than 100 staphylococci/g. The purpose of this study is to determine the feasibility of rapid techniques developed in this laboratory for enumerating the APC, coliforms, E. coli and coagulase-positive staphylococci. Using the proposed methodologies, this complete bacterial quality profile would be obtained in 24 hours.

Several studies into the bacteriological characteristics of frozen breaded shrimp have indicated that excessive numbers of bacteria and quality index bacteria are found in frozen shrimp (Gunderson et al., 1954; Kachikian et al., 1959; Silverman et al., 1961; Nickerson and Pollack, 1973; Vanderzant et al., 1973). The data obtained from the official methods were used to determine if such a pattern still exists. In addition, the data were used to determine the reproducibility of results based on the interlaboratory reports.

REVIEW OF THE LITERATURE

I. Bacteriology of Freshly Harvested Shrimp

The bacteriological characteristics of freshly harvested shrimp have been reported to be important to processors of the raw product. This was first suggested in a broad range, quantitative bacteriological study of freshly harvested and green headed shrimp (Green, 1949a). As early as 1917 (Clark and MacNaughton, 1917) it was reported that shrimp are a perishable food requiring adequate refrigeration and expeditious handling. The early study indicated that freshly caught shrimp should be headed, washed, and iced as quickly as possible because the dark liquid in the stomach concists of oily, partially digested plant and animal material which readily decomposes. It was also suggested that this material be thoroughly removed before washing. DuBois (1946) included the requirement of freezing on board ship in addition to heading and washing to produce the highest possible quality product, while other investigators (Fieger and DuBois, 1946) suggested freezing upon removal of shrimp from the net.

Although the importance of proper handling of freshly harvested shrimp has been recognized since 1917, it was not until thirty years later than an extensive bacteriological

assessment of freshly harvested and green headless shrimp was undertaken (Green, 1949a). The study indicated that the total aerobic bacterial counts of fresh whole shrimp removed from the trawl net varied from 1600 to 1.2 x 10^6/g. It was suggested that because of the location of some samples, an average of 4.2 x 10^4/g was more accurate. The average was compiled from 14 one-shrimp samples caught in commercial fishing nets in widely different areas off the coast of Louisiana. It is of interest that a significant difference was reported between the bacterial load of shrimp within different grades, i.e., number of headless shrimp per pound. According to Green (1949a) an inverse correlation existed between the size of the shrimp and the total aerobic bacterial count. It was also suggested that though the head of the shrimp accounts for only 40% of its weight, it contains up to 75% of the bacterial load. Williams et al. (1952c) published data which supported the preceding study. They estimated the heads of freshly caught shrimp to be responsible for up to 80% of the bacteria in and on the shrimp. Washing the whole shrimp did not significantly reduce the numbers.

It was reported that large numbers of shrimp captured in the Gulf of Mexico were shipped under refrigeration for fresh or frozen processing and that many such shrimp were occasionally exposed to undesirable temperatures and time between capture and the wharf (Williams et al., 1952c). Such reports prompted more quantitative bacteriological

assessments of shrimp at the capture site. Williams et al. (1952c) investigated the bacteriology of Gulf Coast shrimp and reported that the quantitative counts which had been determined, both from individual shrimp and from composites, was too variable to justify conclusions as to the total bacterial load carried by the shrimp. The bacterial counts were reported to range from 3000/g to 2.0×10^6/g. Vanderzant et al. (1970) reported data which closely agreed with that of Williams et al. (1952c). The total aerobic bacterial counts of freshly harvested shrimp from fishing vessels ranged from 870/g to 1.3×10^6/g. Cobb et al. (1973), however, in an extensive study of the bacteriology and chemistry of shrimp from various locations in the Gulf of Mexico, reported that the bacterial counts of fresh shrimp did not exceed 1.0×10^4/g. Green (1949a), in the first quantitative study published, reported bacterial counts ranging from 1600 to 1.2×10^6/g of shrimp from the same area.

According to the data of Harrison and Lee (1968) the bacterial counts of shrimp caught in the Pacific may be comparable to those from the Gulf of Mexico. The investigators reported that such bacterial counts ranged from 3.0×10^5/g to 1.3×10^6/g. The bacterial load of freshly caught shrimp from the Gulf of Maine, however, indicated that temperature may play a role in the nontransient bacteriological flora of fresh shrimp (Zapatka and Bartolomeo, 1973). The total aerobic bacterial counts of the cold water shrimp ranged from 100/g to a maximum of 510/g.

Variation in estimates of the total bacterial count of fresh shrimp were suggested to be due to several factors. These were reported to be intrinsic qualities such as salinity and temperature and extrinsic variables such as the amount of plankton and organic matter present in the area (Cobb et al., 1973). Vanderzant et al. (1970) stated that the number and types of bacteria on shrimp depends upon species, season, fishing grounds, methods of catching, handling on-board, and time temperature of storage. Obviously, there are many factors which can be indicated as variables in the bacterial load of shrimp; however, according to Green (1949a) the bacterial count reflects the differences in handling of the shrimp on the boat, sanitary conditions of the hold and ice, as well as the time and temperature of storage.

Coliforms have been reported by most investigators as not normally present on freshly harvested shrimp. Vanderzant et al. (1970) did not report the recovery of coliforms in their qualitative study of shrimp from the Gulf of Mexico. Green (1949b), however, reported the recovery of the Escherichia coli-Aerobacter aerogenes, presently Enterobacter (Buchanan and Gibbons, 1974), group in 50% of all samples of freshly netted shrimp in the same area. Escherichia coli was only confirmed in one sample. Harrison and Lee (1968) reported the absence of coliforms from shrimp caught in the Pacific, and Zapatka and Bartolomeo (1973) reported similar findings from cold-water shrimp harvested

from the Gulf of Maine. Williams et al. (1952a) and Williams and Rees (1952b) reported the absence of all coliforms in fresh shrimp in the Gulf of Mexico. They suggested that because the volume of their samples was such that conclusions could be drawn, it was their opinion that coliforms of any type are not normally present on fresh shrimp. In addition, coliform numbers in processed shrimp, according to the authors, could be utilized as indication of improper on-board or in-plant sanitation procedures. It is of interest that the microflora of pond-raised shrimp has also been reported not to include any type of coliform (Vanderzant et al., 1971).

The staphylococci, just as the coliforms, are not considered to be of the normal flora of freshly harvested shrimp. Qualitative bacteriological studies by Green (1949b), Williams and Rees (1952b), Williams et al. (1952a), Harrison and Lee (1968), and Cobb et al. (1973) did not include staphylococci in their results. Zapatka and Bartolomeo (1973) reported the absence of Staphylococcus aureus in shrimp from the Gulf of Maine.

II. Bacteriology of Frozen, Raw Breaded Shrimp

To prepare shrimp for breading, green headless shrimp are peeled, deveined, and washed. Processors located in coastal areas frequently use iced shrimp when available from fishing vessels. The peeled and deveined shrimp are spread by hand on a belt which conveys them through liquid batter

and through a heavy sprinkling of a dry breading material. The batter and breading procedure is repeated until the breading approximates 50% of the total weight of the product. Finally, the breaded shrimp are either hand packed on trays for freezing or are placed on trays for overnight freezing prior to packaging.

The procedure has several points where bacteria may gain entrance, increase or be reduced in numbers. Peeling tends to lower the bacterial count substantially as does proper washing (Surkiewicz et al., 1967), whereas during the battering operation and packaging, contamination may occur (Surkiewicz et al., 1967; Liston, 1965). Improper storage conditions allow nontransient and contaminating bacteria to increase in number substantially.

There have been several investigations into the bacteriology of raw breaded shrimp both at the plant and consumer level. Fieger (1950) examined fresh and frozen shrimp and suggested that spoilage was due largely to biochemical changes induced by the microbial population and to a lesser degree, by enzymes and chemical compounds inherent in the shrimp. The first bacteriological analysis of frozen breaded shrimp was reported four years later by Gunderson et al. (1954). These investigators analyzed 27 samples and found a range of 9.5×10^5 to 1.4×10^7 bacteria/g. They also reported having recovered 150 to 1.5×10^4 coliforms/g of frozen raw breaded shrimp and all samples were positive for coagulase-positive Staphylococcus aureus. The authors

considered these data to be of public health significance. Kachikian et al. (1959) reported similar data in their analysis of 144 samples of commercially packed frozen breaded raw shrimp representing 24 different brands and 30 different cities in the United States. The results of the analyses indicated a range in aerobic bacterial counts of 2.1×10^4/g to 5.4×10^7/g of finished product. Thirty-nine percent of the shrimp contained over 1.0×10^6 bacteria/g, while only 7% had plate counts less than 1.0×10^5/g. The numbers of coliform bacteria were reported to vary from 0 to 700/g. Sixty-three percent of the samples contained coliforms, 90% of which were in concentrations greater than 100/g. The authors concurred with the 1954 study in suggesting that the data were far beyond the expected counts and in the interest of public safety, bacteriological standards should be set. Although no definite quality guidelines were offered in the study, it was suggested that in evaluating the sanitary quality of frozen breaded shrimp the aerobic plate count should be used as the primary procedure. Silverman et al. (1961) reported similar data obtained from 56 samples of raw breaded shrimp. In their study, total aerobic counts ranged from 100/g to 7.5×10^6/g. Fifty-nine percent were reported to have counts below 7.5×10^5 bacteria/g. Coliforms were present in 72% of the samples and coagulase-positive Staphylococcus aureus were recovered in 75% of the samples.

The most extensive survey to date on the bacteriology of frozen, raw breaded shrimp was that of Surkiewicz et al.

(1967). The data were based on the inspections of 21 processors of frozen raw breaded shrimp over a 3-year period from which 861 retail packages and 778 line samples were examined. The investigators reported that 13 of the plants were operating under unsanitary conditions. Data compiled from the eight plants considered to have good manufacturing practices were used to establish a profile for the bacteriological quality of frozen, raw breaded shrimp. It was reported that the plants operating under good sanitary procedures produced a product with the following bacteriological characteristics: 1) a geometric average of less than 1.0×10^6 bacteria/g, 2) a geometric average of less than 1000 coliforms/g, 3) the absence of Escherichia coli, and 4) less than 1000 coagulase-positive Staphylococcus aureus/g.

More recent surveys have revealed that despite advances in processing procedures and equipment, there have not been substantial improvements in numbers of total bacteria, coliforms, E. coli, and coagulase-positive staphylococci in the finished, raw breaded product. In such a study, Nickerson and Pollack (1972) reported that in 236 individual breaded shrimp samples collected at the consumer level, 48% had more than 1.0×10^6 bacteria/g. E. coli was reported from 36% of the samples. Vanderzant et al. (1973) reported similar data on in-plant samples. Their data indicated a range of 1.1×10^4 to 6.8×10^6 bacteria/g. Coliforms were present in concentrations between 3.6 and 4600/g with a geometric mean of 66/g. E. coli was isolated from 8%

of the samples at a level of 3 to 3.6/g, and coagulase-positive Staphylococcus aureus were recovered from 50% of the samples. It is of interest that Zapatka and Bartolomeo (1973) found that in plants with poor sanitary conditions, the APC for the finished raw product ranged from 800/g to 2.4×10^5/g with a geometric mean of 1.7×10^4/g. Plants with marginal sanitation practices produced frozen raw breaded shrimp with aerobic plate counts from 100/g to 4800/g with a geometric mean of 850/g. It should be noted that these data were taken from shrimp harvested from plants located near the Gulf of Maine. Thus, the prevailing low temperatures may have had a substantial effect on the bacteriological characteristics of the raw and finished product.

Zapatka and Bartolomeo (1973) reported the the microbial load of raw, frozen breaded shrimp is lower in number than the raw fresh unpeeled shrimp if the processing and sanitation procedures are expeditious and adequate. Although this was true for cold-water shrimp, it has not been the trend for shrimp from the Gulf of Mexico or the Pacific. The APC of the raw product were reported to range from 1600 to 1.2×10^6/g by Green (1949a), 3000 to 2.0×10^6/g by Williams et al. (1952c), 870 to 1.3×10^6/g by Vanderzant et al. (1973), and 3.0×10^4 to 1.3×10^6/g by Harrison and Lee (1968). In comparison, the APC for the finished raw product were reported to be 9.5×10^5 to 14×10^6/g by Gunderson et al. (1954), 2.1×10^4 to 5.4×10^7/g by Kachikian et al. (1957), 100 to 7.5×10^5/g by Silverman et al. (1961), and

1.1×10^4 to 6.8×10^6/g by Nickerson and Pollack (1973). According to the above data, an overall average range of 8.8×10^2 to 1.5×10^6 bacteria/g was reported for fresh, headless green shrimp. A substantially larger average range of 2.7×10^5 to 2.0×10^7 bacteria/g was reported for the finished raw breaded product. In addition to the ranges for APC, coliforms and Staphylococcus are not considered to be part of the normal flora of freshly harvested shrimp (Green, 1949a; Williams et al., 1952a; Williams and Rees, 1952b; Harrison and Lee, 1968; Cobb et al., 1973). It can be assumed then that these "indicator" bacteria are transferred to the product during processing and deserve consideration not only in themselves, but impart a substantial effect on the APC since these bacteria grow well on artificial media.

It is of interest that in the investigations concerning the bacteriology of frozen breaded shrimp, there has been little if any relationship reported between APC and the number of coliforms or E. coli (Nickerson and Pollack, 1972; Silverman et al., 1961; Vanderzant et al., 1973). Such correlations have not been determined for any other possible pair of indicator organisms or APC.

The data of Zapatka and Bartolomeo (1973) indicated that prevailing temperatures may affect the microbial flora of the product. In their study, the shrimp were harvested and processed near the Gulf of Maine. The total bacterial load was reported to be much lower on the fresh and finished breaded product from Maine than on comparable samples from

the Gulf of Mexico. Although this relationship indicated the possibility that such an effect may exist, there has been only one report which suggested that prevailing temperatures may be a real factor in the bacteriological characteristics of frozen raw breaded shrimp. Vanderzant et al. (1973) reported the bacterial counts of raw shrimp used to produce frozen breaded shrimp were highest during the summer months (i.e., July through September). It should be noted that the authors did not report whether these differences were significant.

Surveys undertaken to determine the bacteriological characteristics of frozen breaded shrimp at the retail level and processing level are of interest when they are analyzed. In the investigations of Surkiewicz et al. (1973) and Zapatka and Bartolomeo (1973), the samples were collected from the processor and shipped under dry ice to a central laboratory. Silverman et al. (1961) and Vanderzant et al. (1973) obtained samples from the processing line and returned them to a central laboratory in an unspecified manner. In contrast, Kachikian et al. (1959) and Nickerson and Pollack (1972) collected samples at retail outlets, then packed and shipped them to a central laboratory under dry ice. In all cases the data were the result of assays by a central laboratory. Because there have been no reports of a multi-laboratory analysis of a series of identical samples of frozen raw breaded shrimp, variation in APC, coliform counts and numbers of coagulase-positive Staphylococcus between laboratories

cannot be estimated. Seasonal variation has been largely ignored by the investigators and there was no uniform incubation temperature. Especially noted were the incubation temperatures of the aerobic plate counts. Two were used: 25°C and 35°C. In addition, quantitative procedures for the recovery of coliforms, E. coli and coagulase-positive staphylococci varied.

III. Bacteriological Procedures in the Frozen, Raw Breaded Shrimp Industry

The Aerobic Plate Count

The most widely used microbiological test and according to at least one investigation (Kachikian et al., 1959) the most valuable, is the aerobic plate count (APC). Although Koch is credited with the introduction of the bacterial dilution plate count method, it was not until 1905 that the American Public Health Association recognized the necessity of a standard method for the procedure. In that year a committee was appointed to recommend such a method (Hammer, 1948). The liquid dairy products to which the proposed uniform procedure was to be applied were easily manipulated, and thus their microbiology was becoming understood within the first quarter of the twentieth century. A standard methods manual was published outlining the procedure for the APC for dairy products early in the century (APHA, 1910).

The first bacteriological investigation of meats

reported in the literature as cited by Weinziel and Newton (1914) was reported in the late nineteenth century by St. John. The author reported bacterial numbers associated with poultry products. The problem of uniformity of the technqiue was later recognized by Eyre (1913) and a procedure for the aerobic plate count of meats was published in the second edition of Eyre's Bacteriological Techniques. The publication was not widely accepted as a source of standard procedure, however, and the problem of uniform methodology prevailed. The sentiment of Weinziel and Newton (1914) reflected this problem when they reported that the bacteriological analysis of meat, especially for securing quantitative results, was employed infrequently. In addition, the authors stated that every worker had developed his own procedure with complete disregard for uniformity. As late as 1932 investigators reported that the literature indicated no reference to an accepted standard method for the bacteriological examination of meats (Geer et al., 1932). During this period, consumer acceptance of frozen food increased and many investigators expressed concern about the lack of knowledge for the bacterial assessment of the frozen products.

The underlying problem in the analysis of meats was the method of homogenizing the sample for dilution and plating, or whether to do so at all. As Geer et al. (1932) noted, "the more thoroughly the tissue is disintegrated, and the clumps of bacteria broken up, the nearer the plate count

will be to the actual number present." Such procedures consisted of macerating the meat with a mortar and pestle and sterile sand (Weinziel and Newton, 1914), a method suggested originally by Russell and Weinziel (1897), then washing with a known amount of sterile diluent and plating. Another procedure required shaking a small sample with sterile sand or glass or both and plating (Weinziel and Newton, 1914), while other procedures did not suggest the need for homogenization at all (Eyre, 1913). The need for uniform methodology was aptly put by Hunter (1934) in expressing his concern about the processing of crustacea. He suggested that the technical control of preparation and legal control of production, distribution, and sale involve bacterial analysis. "Thus, for the accomplishment of the greatest good there should be one method of analysis acceptable to and used in common by all agencies having a part in the sanitary control of the products."

Although now established as a usable quality criterion in the seafood industry, it is doubtful that another technical procedure has survived the testing and criticism of the aerobic plate count. Wright and Thorton (1927) reported the reproducibility of the procedure in the bacterial analysis of milk, stating that with the same medium, the same methods, and the same operator, a variation of less than 100% within a series of the same sample cannot be depended upon. They judged the plate count inaccurate and statistically useless. A similar study was undertaken by Mudge

and Lawler (1928) who reported data from plating nine samples of milk in a single series using 75 plates per series and the same operator. The results of the investigation were reported to be "unimpressive." The data indicated that multiplication of the bacteria during diluting and plating might have been an important factor to which further studies should be directed.

Prior to 1932 assessments of the reproducibility of the APC technique were performed on the same product in series using the same operator. However, in that year Schacht and Robertson (1932) reported the results of four plate counting contests between laboratories of different cities using identical samples. Thirty-five individuals counted one or more plates, one or more of seventeen different combinations of counting were used, and a total of 38 plates were counted and 1543 duplicate counts recorded. The data indicated that the "personal equation" offers the greatest source of error because only in six of the 38 plates were the percentage differences among individuals' means less than 15% and 37% of those were greater than 30%. They also noted very wide differences among some individuals and extremely small differences between others when their duplicate counts, using the same devices on the same plate, were compaged. The major causes of error in the plate counts were given as: 1) failure to know what to count, 2) failure to see colonies, and 3) carelessness.

Several investigators have suggested that the

reproducibility of the APC decreases with an increase in the number of bacteria in the sample being tested. Tiedman (1933) reported plate counts made by different laboratories on identical samples of low count raw milk were in close agreement. Aerobic plate counts made by the same laboratories on high count raw milk and abnormal milk, however, showed considerable variation. In a report cited by Davis (1969a), minced meat was artificially contaminated and sent to several different laboratories. The results were again in close agreement until high-count samples were used. Davis (1969b) also reported that a competing flora may effect variation in the aerobic plate count.

Other factors have been attributed to the variation in the aerobic plate count. Mudge and Lawler (1928) reported that multiplication during diluting and plating affected the accuracy of the procedure, while Jennison (1937) reported that clumping of bacteria may be a major factor. Jennison and Wadsworth (1940), in a statistical study of the sampling error and dilution error (i.e., errors in pipetting in reaching a given dilution), reported that although the former was thought to be more important by most investigators, the latter was at least as important, if not more so. Davis (1971) suggested that other factors ultimately affecting the reproducibility of the aerobic plate count were: 1) immediate treatment, 2) method of transport, 3) holding the sample in the laboratory, and 4) all aspects of the methods used, i.e., quantity used in enrichments, media, and

temperature of incubation. It is of interest that duration of incubation was not considered.

It should be mentioned that even in consideration of the reported variables in the APC procedure, additional ones have been noted when the technique is applied to fish and other seafood products. ZoBell and Conn (1940) observed that melted, warm agar temperatures decreased microbial recovery when pour plates were used. The majority of the marine microorganisms were killed when exposed to 30°C to 40°C for 10 minutes. Findings of Klein and Shenyuh (1974) supported the previous study, wherein the investigators demonstrated a highly significant difference between results of pour plate and spread plate techniques when applied to heterotrophic aquatic microorganisms. They suggested that differences in the counts were due to the exposure of the bacteria to the molten agar in the plate count technique. The temperature of the agar results in a stress on the bacteria which reduces their numbers substantially.

Although reports of questionable reproducibility are extant in the literature, the APC is currently one of the principal tests of quality in the seafood industry. However, recognition of its limitations have been suggested by most investigators. Jennison and Wadsworth (1940) stressed that such a count established bacterial numbers for comparative purposes only if relative rather than absolute numbers of cells are reported. Corlett (1974) suggested that if the APC were used correctly, it could be useful in determining

the effects of harvesting, storage, processing, preservation, and packaging. The technique has been reported useful in such situations since prior to onset of organoleptic deterioration, counts must be in the order of 10^6 bacteria/g. This was reported to be true in protein foods by Elliot and Michener (1961).

Most Probable Number (MPN) of Coliforms and Escherichia coli

Use of other bacteriological quality tests in foods important to the frozen shrimp industry are the coliforms, including E. coli. The first reported use of E. coli as a possible quality criterion was in 1880, wherein Clark and Kabler (1964) cited Von Fritsch as the first proponent of the use of the bacterium for the potability assessment of water. Five years later, Escherich noted two species of bacteria which he established as nontransients of human feces. The organisms were later classified in the genera Escherichia and Aerobacter (Enterobacter). He further suggested that the presence of these bacteria in water constitutes a hazard since fecal pollution may be implicated. It was assumed that their presence indicated an increased probability of enteric pathogens introduced into the water by infected persons just as were the nontransient coliforms.

As work progressed into the nature of coliforms, the organisms were separated into two groups, fecal and nonfecal, a technical classification that stemmed from the early work of Eijkman (1904). The investigator suggested

separation of fecal and non-fecal coliforms, regardless of recovery environment, by incubation at elevated temperatures. According to Eijkman, coliforms of public health significance produced gas from fermentable carbohydrates at 46°C while others did not. However, the elevated temperature technique was not immediately accepted. Parr (1939) and Kaufmann (1951) emphasized that no single laboratory procedure could differentiate between coliforms of fecal and non-fecal origin. Parr (1939) suggested several additional tests be run, including indole, methyl red, Voges Proskauer, and citrate utilization. Although this battery of tests is preferred in pure culture studies, it has been shown they are not required in routine quality assessment tests. Hajna and Perry (1935) reported that a modification of Eijkman's elevated temperature test for fecal coliforms using a special medium did well in estimating concentrations. The investigators also demonstrated that the increased temperature had no cultural effect on Escherichia coli. Fishbein et al. (1967), using Lauryl Tryptose broth, suggested a 24-hour elevated temperature procedure for the recovery of E. coli from foods.

Largely through the work of Griffin and Stuart (1940) E. coli has been accepted as the valid coliform of fecal origin. In a broad environmental study, the authors isolated over 6000 coliforms from milk, water, soil, grains, and feces. E. coli was isolated consistently from feces but only rarely from other environments. In addition, the other coliforms were rarely recovered from feces. E. coli was

suggested as the coliform of public health significance.

It is currently accepted that not only is E. coli of public health significance when recovered from foods, but that the non-fecal coliforms are suitable indices of poor processing procedures and sanitation habits in many food products. Just as Sherman and Wing (1933), Thomas (1955), and others have reported the test for non-fecal coliforms to be a valuable index of proper processing of milk. This also has become a standard test in the processing and quality assessment in the seafood industry.

Most Probable Number (MPN) of Coagulase-Positive Staphylococci

The most recently established indicator organisms in the seafood industry are the coagulase-positive staphylococci. The first reported case of staphylococcal food poisoning probably appeared in 1884, the same year in which Rosenbach described the type species for the genus Staphylococcus (Breed et al., 1957). In that year, 300 cases of severe illness attributed to the consumption of cheddar cheese were tabulated (Vaughn, 1884). Vaughn ingested material he extracted from the cheese and exhibited comparable symptoms. After microscopic examination of the cheese, he concluded that spherical bacteria produced a chemical in the food which rendered it toxic. In 1914 the first well documented case of staphylococcal food intoxication was reported by Barber, who investigated a milk-born food poisoning outbreak in the Philippines and consistently isolated

staphylococci from the incriminated cows. Dack et al. (1930) isolated a yellow Staphylococcus in large numbers from cake which had been a suspect food responsible for an outbreak of poisoning. Sterile filtrates of broths used to culture the bacteria were administered to human volunteers and the symptoms were reproduced (Dolman and Wilson, 1938). These outbreaks and others reported in the literature were reviewed by Jordan (1931). The author suggested that the staphylococci were responsible for a newly found type of food poisoning. It is of interest that staphylococcal food poisoning has been reported as the most common type of food intoxication. Olsen (1968) speculated that there are several million cases of Staphylococcus food poisonings annually in the United States.

Not all the staphylococci are capable of producing toxin in foods, and several physiological characteristics were suggested as indices of enterotoxigenicity. Although Feldman (1946) suggested hemolysin production as an index of enterotoxin production, an earlier suggestion by Chapman (1944) for production of coagulase as an indication of enterotoxigenicity has been more widely accepted. Studies by Evans and Niven (1950) implied that most, if not all, food-poisoning staphylococci are coagulase positive. Bergdoll et al. (1967) and Thatcher and Simon (1956), however, reported four isolates which were coagulase-negative yet still produced enterotoxin.

Fermentation of mannitol has also been reported as a

possible index of enterotoxigenicity. Gwatkin (1937) reported that mannitol was fermented by 190 of 275 strains of staphylococci which caused mastitis in cows. Joshi and Dale (1963) suggested mannitol fermentation as a reliable method for detection of coagulase-positive Staphylococcus provided anaerobic conditions were maintained. Zemelman and Longeri (1965) reported that of 404 coagulase-positive staphylococci isolated from bovine raw milk, 92% fermented mannitol.

In a recent review, Minor and Marth (1971) suggested that there is no single physiological characteristic or combination of characteristics known to be possessed by the staphylococci which is an absolutely reliable indicator of enterotoxigenicity. However, currently the consensus is that coagulase positivity is the most suitable criterion.

Rapid Techniques for APC, Coliforms, E. Coli, and Coagulase-Positive Staphylococci

The present methods used to determine the bacteriological profile of in-line or finished product in the frozen raw breaded shrimp industry are essentially those used in all other food industries. The accepted procedures for the aerobic plate count are outlined in several publications. Although the techniques vary in their titles, i.e., "standard methods" (APHA, 1965, 1967), "recommended methods" (APHA, 1966), or "official procedures" (AOAC, 1970), the procedures are essentially identical. The solid food is homogenized in phosphate-buffered diluent similar to that described by

Butterfield (1932), diluted in the same diluent, plated out in decimal aliquots in Plate Count agar, and incubated at 32°C (APHA, 1965), 35°C or 20°C (APHA, 1967), or 35°C (AOAC, 1970) for 48 hours.

Although Plate Count agar is the accepted medium for the aerobic plate count, Eugon agar has been reported to yield higher counts with seafoods (Nickerson and Sinskey, 1972). This was attributed to the greater availability of dextrose and the resultant increase in metabolic rate of the organisms. Another point of interest has been the complete acceptance of the 48-hour incubation period. Although longer incubation times have been suggested for foods containing a microflora dominated by psychrophiles or psychrotrophs, or for techniques designed to enumerate these organisms exclusively (Nelson and Barder, 1954; Vanderzant and Moore, 1955), an incubation period of less than 48 hours is not suggested for routine plating procedures.

The procedure outlined in the Bacteriological Analytical Manual for Foods (U.S. Dept. of Health, Education, and Welfare, 1969) and Association of Official Analytical Chemists (AOAC, 1970) for enumeration of coliforms requires even more time and media than does the aerobic plate count procedure. Basically, the method is the Most Probable Number (MPN) procedure introduced and improved by Hoskins (1933, 1934). The official procedure entails two separate MPN procedures using two different broths. From the time of homogenization of the sample, 4 days are required before the

results are known.

Similar to the procedure for the enumeration of total coliforms, the analysis of foods for Escherichia coli, or "fecal coliforms," also is an MPN procedure. The technique is described along with that of the coliforms (AOAC, 1970; U.S. Dept. of Health, Education, and Welfare, 1969). The method specifies three broths and one solid medium and may require up to 7 days for completion.

An official technique (U.S. Dept. of Health, Education, and Welfare, 1969) is listed, however, which requires only 24 hours incubation time for the MPN. This is essentially an elevated temperature method similar to that described by Fishbein et al. (1967). Since plating on differential agar is needed, the procedure requires a total of 48 hours.

There are at present two official methods for the enumeration of coagulase-positive Staphylococcus aureus. BAM (U.S. Dept. of Health, Education, and Welfare, 1969) described a direct plating method using the medium introduced by Baird-Parker (1962). The procedure is a spread technique in which typical colonies are counted and tested for coagulase production. Although the procedure is relatively rapid, it is not recommended for foods with greater than 100 coagulase positive staphylococci per gram. This restriction may prove to decrease the value of the technique in the frozen, raw breaded shrimp industry. The second method, requiring a minimum of 4 days, is essentially a 3 x 3 MPN procedure in

selective broth and differential agar. Typical colonies are picked from the agar plates and tested for the production of coagulase.

Foster et al. (1970) described a method recommended by the Food Research Institute (University of Wisconsin). The procedure is similar to the official direct plating method and has the same disadvantage of limited recovery. Thatcher and Clark (1968) also reported direct plating methods, suggesting five procedures employing Baird-Parker, Egg Yolk-Azide, Milk Salt, or Tellurite-Polymyxin-Egg Yolk agars and one method requiring enrichment in a selective broth prior to plating on differential media.

Processing techniques in the frozen raw breaded shrimp industry have improved so rapidly that the finished product is on the retail shelf in less time, thus offering a fresher product to the consumer. However, bacteriological tests used to measure the quality of the raw and finished product have not changed the time required for their performance. Guidelines set by the regulatory agencies are for the protection of the consumer, and the official bacteriological testing procedures, when carried out properly, impart a good measure of the relative quality of the product. These procedures, though, are not compatible with processing techniques in that the time requirement is such that processing must be delayed if the final bacteriological data are to be analyzed before shipment. Rapid, reliable bacteriological tests, not to replace official procedures but to supplement

them, are clearly needed in the frozen seafood industry.

Rapid procedures for bacteriological analysis in foods are not new concepts. Frost (1912, 1921) suggested a technique which became known as the "little plate method" in which inoculated agar drops were allowed to solidify on microscope slides. The slides were incubated for 4 hours and resulting colonies counted under low power. The Frost method was reported to be comparable to those of the standard aerobic plate count procedures for the examination of dairy products (Hatfield and Park, 1922). Winter et al. (1971) used a procedure comparable to the little plate method, however, the convenience of membrane filters was exploited. The authors reported that by rinsing microbial cells from foods and concentrating the diluent on the surface of the membrane filter quantitatively, a total bacterial count could be performed in 4 to 6 hours. The "seeded" membrane filters were incubated on absorbent pads saturated with nutrient broth, fixed in janus green, and the resultant microcolonies counted under low power.

The most recent suggestion of rapid methodology for the aerobic plate count was that of Sharpe et al. (1972) and Sharpe and Kilsby (1972) who described their procedure as "agar droplets." A sample suspension of 0.1 ml quantitatively in molten agar was placed in petri plates. The resulting plates were incubated at 35°C and the microcolonies counted after 8 hours. With the development of additional accessories for the droplet procedure (Sharpe et al., 1972),

the investigators suggested that the technique would be ideal for quality control in the food industry.

Rapid procedures for quantitative recovery of coliforms and E. coli from foods have been reported by several investigators. Methods which involve simply plugging the fermentation tubes with stoppers such as that suggested by Davis (1939) have been suggested. Davis suggested that the gas produced from the fermentable carbohydrate was more readily noted if stoppers were used. Semi-solid media were employed by Bicknell et al. (1952) in which they reported trapped gas could be observed more rapidly than the conventional Durham tube. Moldovan (1935, 1965) reported that an agar overlay allowed all of the gas to be trapped rather than that produced at or below the Durham tube opening. The author reported more rapid and greater recovery of coliforms from dairy products using the overlay technique. Stuart and Weaver (1948) suggested that the agar overlay was at least as sensitive as the Durham tube, and McDade and Weaver (1959), after studying several rapid methods, suggested that the agar overlay was more sensitive than the conventional Durham tube. Kendall (1973) suggested that with the use of massive inocula, coliform and E. coli MPN procedures could be determined in less than 12 hours. An agar-plugged inverted tube technique was described which was required for the rapid recovery.

Labeled carbohydrate techniques have been reported by Korsch (1970), making detection of E. coli possible in 4 hours. The method did not prove to be sensitive enough for

small numbers of organisms, however. Levine et al. (1957) reported even more rapid results by using I-C^{14} lactose as the carbohydrate source, wherein radioactive carbon dioxide could be detected within one hour.

Despite many suggested rapid procedures, the Durham tube method is the most widely used. Its simplicity and relative low cost compared with other tests have made it difficult to replace. Fishbein et al. (1967) described a rapid procedure for recovery of E. coli using a Durham tube MPN procedure. The method described a simple one in which a 3 x 3 MPN is performed in LST at 44.5°C for 24 hours. LST was preferred over the official EC broth because it promoted greater cell recovery (Fishbein, 1962).

Minor and Marth (1971) suggested the need for more rapid techniques for the detection of coagulase-positive Staphylococcus aureus in their review of present methods of enumeration. Unfortunately, the literature shows little work in this area.

Wilson et al. (1959) described a procedure in which 20 grams of sample were blended in 99 ml of phosphate buffer. From this homogenate, 0.5 ml was transferred to 1 ml of BHI containing 2% mannitol and 7.5% NaCl and incubated at 35°C with shaking for 4 to 6 hours. The broth was examined for gram-positive organisms and a coagulase test performed on typical isolates. A more rapid procedure, requiring only 3 hours, was proposed by Chesbro and Auborn (1967). The authors observed that Staphylococcus aureus produced a stable

deoxyribonuclease in any conditions which allowed growth. They extracted the nuclease from the food sample and measured it quantitatively. The method was based on the assumption that enterotoxigenic staphylococci produce the enzyme while others do not. According to Lachica et al. (1971), 95% of 251 enterotoxin-producing strains studied produced the nuclease and 93% of those strains were coagulase positive. Similar results were reported by Brandish and Willis (1970) and by Lachica et al. (1969).

EXPERIMENTAL PROCEDURE

I. Methods

This investigation originated out of a cooperative of the quality control laboratories of 10 processors of frozen, raw breaded shrimp, breading producers, FDA laboratories, and the bacteriology laboratory of the Department of Food Science at Louisiana State University. Procedures were developed by the LSU investigators for the aerobic plate count, total coliforms, and coagulase-positive staphylococci that would require only 24 hours for all tests. In addition, the 24-hour test described by Fishbein et al. (1967) for E. coli was incorporated in the study. The source of the samples were processors of raw frozen breaded shrimp (a detailed list is on deposit at the LSU Department of Food Science) who sent their final product to the University fully packaged.

Each month a processor shipped a sample of his product to the Food Science Department. The source of the sample varied in location throughout the southern tier of the United States, including Georgia, Florida, Texas, and California. The sample was fully packaged and identical to that found in retail outlets. The sample was packed under dry ice for shipment in boxes designed and marked for such shipment. Upon arrival, the frozen raw breaded shrimp samples were

thoroughly inspected for signs of thawing. Such samples were discarded and new ones requested. After the inspection, the packaged breaded shrimp were placed in a -20°C freezer for approximately 24 hours.

After storage a 10^{-1} dilution was prepared by using Butterfield's buffer with an appropriate amount of breaded shrimp. Homogenization was accomplished in a sterile stainless steel blender jar designed for the commercial Waring Blendor capable of yielding 3 liters of homogenate. The sample was homogenized for 2 minutes and the resulting homogenate was transferred aseptically to sterile 100 ml polyethylene bottles, capped and returned to the freezer.

The following day duplicate frozen, bottled homogenates were placed in small polystyrene buckets with approximately 5 pounds of dry ice, sufficient to maintain the frozen state for 48 hours, the tops of the buckets taped and placed in insulated boxes designed for dry ice shipment of perishable items. One duplicate set was retained at -20°C for analysis at the LSU laboratory. All bottles were labeled and coded so that only the investigators at LSU knew the processor from which the sample originated. The code on each bottle described the source of the sample, the date shipped by the processor, the laboratory to which LSU shipped the prepared sample, and the date of that shipment, though not in that order. The frozen samples were shipped to the 16 analytical laboratories and the data were reported only to the LSU investigators.

Upon receipt by the participating laboratories the samples were inspected and placed in commercial freezers. Basically, the raw breaded shrimp were shipped to LSU by one of 10 processors, homogenized by LSU investigators to yield a 10^{-1} dilution, frozen, shipped with dry ice to all participating laboratories, and placed in the commercial freezers. The following Monday (4 days after shipment from LSU) all laboratories initiated the bacteriological analysis of the homogenates.

The first sample of frozen breaded shrimp was received by LSU in December 1970. Seventeen additional samples were studied including a second in December 1970 and one in January 1971; one in March 1971; three in June 1971; and one in August 1971. Three shipments were made in 1972, in the months of June, August, and October. Four shipments were made in 1973 including two in February and one each in May and October; one was made in February of 1975. The data sent to the Department of Food Science by the analytical laboratories are listed in Tables A and B (Appendix).

Methods of Analysis: AOAC versus Rapid Methodology

Two methods were used for the enumeration of APC, coliforms, *E. coli*, and coagulase-positive staphylococci by the 16 analytical laboratories. The standard reference was those official procedures found in *Official Methods of the Association of Official Analytical Chemists* (AOAC, 1970). The second method was a series of rapid procedures utilized and

developed by the Department of Food Science, LSU.

The Aerobic Plate Count

AOAC: Plate Count agar was used in the procedure. Dilutions were prepared using Butterfield's buffer. The dilutions used were 10^{-1} to 10^{-6} in duplicate. The sterile agar was held at 45°-47°C and poured at that temperature. The agar was allowed to solidify, inverted and incubated for 48 hours at 35°C. The resulting colonies were counted on a Quebec colony counter using standard counting procedure.

Rapid Method: Eugon agar was used in this procedure. Dilutions were prepared in Butterfield's phosphate buffer. The dilutions used were 10^{-1} to 10^{-6} in duplicate. The sterile agar was held at 45°-47°C and poured at that temperature. The plates were allowed to solidify, inverted, and incubated at 35°C for 24 hours. The resulting colonies were counted on a Quebec colony counter.

Total Coliforms

AOAC: A 3 x 3 MPN using LST broth and dilutions of 0.1, 0.01, and 0.001 was incubated at 35°C for 48 hours. Gassing tubes were then transferred to BGLB 2% using a 3mm loop. The BGLB tubes were set up to retain the 3 x 3 MPN pattern. These tubes were incubated at 35°C for another 48 hours. MPN codes were taken from the positive BGLB tubes after the incubation period and their numbers recorded.

Rapid Method: A 3 x 3 MPN using LST broth and dilutions of 0.1, 0.01, and 0.001 was incubated at 35°C for 24

hours. MPN codes were taken from the gassing tubes after the incubation period and their numbers recorded.

Escherichia coli

AOAC: Gassing tubes from the LST MPN of the AOAC coliform procedure were used to inoculate EC broth to retain the 3 x 3 MPN pattern. The EC broth was incubated at 44.5°C for 48 hours. Growth from positive tubes were used to streak EMB agar. Typical colonies were noted and a MPN code taken from the plates after an incubation period of 24 hours at 35°C.

Rapid Method: A 3 x 3 MPN using LST broth and dilutions of 0.1, 0.01, and 0.001 was incubated in a 44.5°C water bath for 24 hours. MPN codes were taken from the positive tubes after the incubation period.

Coagulase-Positive Staphylococci

AOAC: A 3 x 3 MPN using Trypticase Soy broth and dilutions of 0.1, 0.01, and 0.001 was incubated at 37°C in a water bath for 48 hours. After the incubation period, positive tubes were transferred to Vogel-Johnson agar and incubated for 24 hours at 35°C. Typical colonies were picked from the plates and streaked onto nutrient agar slants which were incubated for 18 hours. This growth was used to inoculate 0.2 ml of Brain Heart Infusion broth with incubation for another 18 hours. After the incubation period, 0.5 ml of rehydrated coagulase plasma EDTA was added to the tubes and incubated for 4 hours at 37°C. Tubes showing coagulated

plasma were recorded and used to determine the final MPN of coagulase-positive staphylococci.

Rapid Method: A 3 x 3 MPN using TMM broth and dilutions of 0.1, 0.01, and 0.001 was incubated for 24 hours in a 37°C water bath. Tubes indicating an acid reaction by the phenol red indicator were considered positive and used to establish the code for the MPN table listed in the AOAC manual (AOAC, 1970).

II. Materials

Media and Reagents

The composition of all media used in this investigation is listed in Tables C through L in the Appendix. All media were sterilized by autoclaving at 121°C for 15 minutes.

Butterfield's Phosphate Buffer

All dilutions were made with Butterfield's phosphate buffer (Butterfield, 1932). A stock solution was prepared by dissolving 24 grams of potassium acid phosphate (KH_2PO_4) in 500 ml of distilled water. The pH was adjusted to 7.2 with 1N NaOH and the mixture diluted to one liter with distilled water. The stock solution was stored at 4°C until needed. Diluents were prepared by adding 1.25 ml of the stock solution to one liter of distilled water and adjusting the pH to 7.2 with 0.1N NaOH. The buffer was sterilized by autoclaving at 121°C for 15 minutes.

Aerobic Plate Count

Plate Count Agar is the standard medium for the enumeration of bacteria in milk, water, waste water, and foods (APHA, 1965, 1966, 1967; AOAC, 1970). The medium was prepared by dissolving 24 grams in 1000 ml of distilled water.

Eugon agar has been reported to yield higher recovery of bacteria from seafoods (Nickerson and Sinskey, 1972), mainly attributed to the greater availability of dextrose which allowed more rapid colony production than standard Plate Count agar. Thus, Eugon agar was chosen as the medium for the rapid aerobic plate count technique. To rehydrate the medium 45.5 grams were dissolved in 1000 ml of distilled water (Difco, 1953).

Total Coliforms and Escherichia coli

Lauryl Tryptose Broth (LST) is the standard medium for the presumptive determination of coliforms in water, waste water, and foods (APHA 1965, 1966, 1967; AOAC, 1970). Tubes from the MPN procedures showing gas production were transferred from the LST to EC medium. The EC medium is recommended for the MPN procedure in the determination of E. coli.

It has been reported that LST is less inhibitory than EC, thus yielding greater recovery of E. coli at elevated temperatures (Fishbein et al., 1967). LST was chosen as the MPN medium for the rapid procedure for E. coli, and was

retained for the rapid test for total coliforms. Preparation of the medium was prepared by dissolving 35.6 grams of the dehydrated medium in 1000 ml of distilled water. EC medium is recommended as a secondary confirmatory medium in the examination of water, waste water, and foods for E. coli when used at 44.5 or 45.5°C (AHPA, 1965, 1966, 1967; AOAC, 1970). EC medium was prepared by dissolving 37 grams of dehydrated powder in 1000 ml of distilled water (Difco, 1953).

Brilliant Green Lactose Bile (2%) (BGLB) is the confirmatory medium for the determination of coliforms in water, waste water, and foods (APHA, 1965, 1966, 1967; AOAC, 1970). Primary LST MPN tubes showing gas production after 48 hours are used as inocula in BGLB. To prepare the medium, 40 grams of dehydrated powder were dissolved in 1000 ml of distilled water (Difco, 1953).

Eosine Methylene Blue agar is recommended as a confirmatory differential plating medium for E. coli (AOAC, 1970). It is suggested that growth from the elevated temperature MPN positive tubes be streaked onto EMB and typical E. coli colonies counted. The medium is prepared by dissolving 37.5 grams of dehydrate into 1000 ml of distilled water and autoclaving (Difco, 1953).

Coagulase-Positive Staphylococci

Trypticase Soy broth is recommended as the MPN medium for enumeration of coagulase-positive staphylococci in the official procedure (AOAC, 1970). To increase the selectivity of the medium, 10% NaCl is added. To prepare the medium, 30 grams of the dehydrate are dissolved in 1000 ml of distilled water and dispensed in 9-ml amounts in screwcap tubes.

Vogel-Johnson agar is recommended for isolation of coagulase-positive, mannitol fermenting staphylococci from foods (AOAC, 1970). The medium is used as a secondary MPN procedure in conjunction with trypticase soy broth. Typical colonies are produced by mannitol fermenting staphylococci capable of reducing potassium tellurite. To prepare the medium, 60 grams of dehydrate are dissolved in 1000 ml of distilled water and autoclaved (Difco, 1953).

Tryptic Mannitol Meat broth (TMM) was chosen for the 24-hour MPN of coagulase-positive staphylococci due to its ability to differentiate mannitol-positive staphylococci from negative strains. Several workers have reported that the fermentation of mannitol by Staphylococcus is a reliable indication of enterotoxigenicity and coagulase positivity, especially under low oxygen tension or anaerobiosis (Gwatkin, 1937; Joshi and Dale, 1963; Zemelman and Longeri, 1965). The TMM was dispensed into screw-cap tubes and autoclaved, after which they were tightened while hot to maintain low oxygen concentrations. Phenol red was added to indicate

acid production from the fermentation of mannitol with 10% NaCl and Polymyxin B as selective agents. The medium was prepared by dissolving 46 grams of the dehydrate in 180 ml of distilled water. Commercially available Polymyxin B solution was added to yield a final measure of 7,500 units per 9-ml tube. The medium was dispensed in the screw-cap tubes and autoclaved.

Brain Heart Infusion (BHI) is a broth especially designed for cultivation of fastidious pathogenic bacteria. It is a recommended official procedure (AOAC, 1970) as the final culture medium for isolates prior to testing for coagulase production. To prepare the broth, 37 grams of dehydrate are dissolved in 1000 ml of distilled water (Difco, 1953).

RESULTS AND DISCUSSION

Data from the interlaboratory bacteriological procedures for estimating numbers of total bacteria (APC), Escherichia coli, coliforms, and coagulase-positive staphylococci in raw frozen breaded shrimp are presented in Tables A and B in the Appendix. The data collected from the 16 analytical laboratories represent 18 replicate samples of raw breaded shrimp sent to the Louisiana State University Department of Food Science by one of 10 different sources per month. The sources were of various locations within the southern tier of the United States from Florida to California, and shipments of their samples continued over a period of 4 years.

Sixteen laboratories cooperated in compilation and comparison of bacterial counts of identical series of samples. As a result, 2125 units of data were gathered. The duplicate results sent to the Department of Food Science, LSU, by the participating laboratories were averaged and a total of 1349 observations was recorded. Of these, 676 were the result of the official procedures of analysis, while the remaining 673 observations were from the proposed rapid, monitoring techniques. The data were key-punched and evaluated at the LSU Computer Research Center using the Statistical Analysis System (SAS) developed by Goodnight and Barr (1971).

The data gathered for each each bacteriological analysis, aerobic plate count (APC), total coliforms, E. coli, and coagulase-positive staphylococci, were subjected to the least means squares analysis in which were examined the effects of source, laboratory, technique, and the interaction, laboratory x technique on the adjusted mean values for the observations of the four different types of analyses. Results of the analysis of variance are presented in Tables M-P (Appendix).

Aerobic Plate Count of Frozen Raw Breaded Shrimp

During the entire analytical period, December 1970 through February 1975, the mean APC values for the 10 different sources (processors) ranged from 2.7×10^4 to 1.0×10^6/g with an overall mean of 6.9×10^5 bacteria/g. These data included all samples sent to the analytical laboratories using both the official methods of analysis (AOAC, 1970) and the proposed rapid procedures.

The individual mean values reported by the analytical laboratories for the 10 different sources are listed in Table I. Data are arranged in descending order of magnitude of the mean values for each source of raw breaded shrimp, together with the number of observations per source reported by the various analytical laboratories. The mean APC values for source varied markedly with a range of 2.7×10^5 to 2.7×10^7/g. This corresponded roughly to a 100-fold difference.

Table I. Mean APC for 10 Sources of Frozen Raw Breaded Shrimp Reported by 16 Analytical Laboratories

Rank	Source Code Number	Observations per Source	Mean APC/g ($\times 10^4$)
1	10	20	272.9
2	1	21	109.3
3	5	36	73.7
4	3	70	68.5
5	6	52	41.2
6	7	32	39.9
7	8	19	38.7
8	2	33	28.3
9	9	14	21.7
10	4	37	2.7

These differences between sources were not unexpected. The processors were in various parts of the southern tier of the United States with varying climatic conditions. In addition, the raw shrimp used to produce the finished frozen breaded product varied as to location of capture. Such factors acted to produce data indicating that frozen raw breaded shrimp from different geographical areas vary widely in their bacteriological quality characteristics.

The mean APC values associated with the 16 reporting laboratories ranged from 6.8×10^4 to 1.4×10^6 bacteria/g, corresponding roughly to a 20-fold difference between extremes. The individual mean APC values associated with the analytical laboratories are presented in Table II. The F value for laboratories was 1.50, which was not significant ($p > 0.05$). The critical values for d.f. 15/284 were $F_{.05} = 1.67$ and $F_{.01} = 2.04$.

Table II. Mean APC Values for Frozen Raw Breaded Shrimp Reported by 16 Analytical Laboratories

Laboratory Code Number	Observations per Laboratory	Mean APC/g ($\times 10^4$)
1	4	6.8
2	26	82.3
3	17	82.7
4	18	36.5
5	28	62.5
6	36	63.7
7	32	77.1
8	20	9.3
9	14	141.3
10	15	71.5
11	36	44.7
12	30	74.1
13	30	33.7
14	8	101.7
15	16	63.5
16	4	11.3
Overall	334	60.16

The finding of no significant difference between the laboratories for the APC of the samples is not in agreement with previous reports, although such investigations were not undertaken with seafoods. According to Wright and Thorton (1927) using the same medium, method, and operator, a variation in the total plate count in a series of the same sample of less than 100% could not be expected. In other investigations of the reproducibility of aerobic plate counts, similar conclusions have been reported (Mudge and Lawler, 1932; Tiedman, 1933; Jennison and Wadsworth, 1940). In interlaboratory studies, Davis (1969a) cited an investigation which produced marked variation in results of APC when minced meat was artificially contaminated. Schacht and

Robertson (1932) in an interlaboratory investigation on the reproducibility of APC data reported a large variation in counts using a series of the same sample not only between cooperating laboratories, but between operators in the same laboratory as well.

Table II indicated that variation existed between laboratories in the APC analysis of frozen breaded shrimp. However, the variation in individual samples necessitated large variation between means to produce a significant ($p < 0.05$) F value.

The mean APC values associated with the official and rapid techniques reported by the analytical laboratories are listed in Table III.

At 13 of the analytical laboratories the official technique yielded greater mean values than the rapid technique. The interpretation of this was a greater recovery of bacteria, because of the greater incubation time, yielded the larger means. The F value for technique was 0.41 which was not significant ($p > 0.05$). The critical values for F at d.f. 1/284 were $F_{.05} = 3.84$ and $F_{.01} = 6.63$. The F value for the interaction, technique x laboratory was 0.14 which was not significant ($p > 0.05$). This indicated that with respect to the mean APC values for the samples of frozen breaded shrimp, the relationship between laboratories and techniques was consistently maintained for all samples.

Table III. Mean APC Values and Number of Observations Reported by the Analytical Laboratories Using the Official and Rapid Techniques

Laboratory Code Number	Official Technique		Rapid Technique	
	Observations per Laboratory	Mean APC ($\times 10^4$)	Observations per Laboratory	Mean APC ($\times 10^4$)
1	2	8.4	2	5.2
2	15	80.1	11	85.4(R)
3	9	78.0	8	88.0(R)
4	9	40.1	9	32.9
5	14	77.5	14	47.5
6	18	71.7	18	55.7
7	16	87.7	16	66.6
8	9	9.9	11	8.8
9	7	164.2	7	118.4
10	8	90.8	7	49.4
11	18	50.3	18	39.1
12	15	75.2	15	73.0
13	15	39.9	15	27.5
14	5	94.2	3	113.6(R)
15	8	68.0	8	59.0
16	2	12.0	2	10.5
Overall	170	65.5	164	54.7

(R) = Relationship between techniques reverse of that for the overall means.

The data indicated that there was no significant difference ($p > 0.05$) between the official (AOAC, 1970) and rapid techniques. In addition, the demonstration that a consistent relationship existed between the 16 laboratories using the rapid technique would suggest a useful application of the technique in the food industry. The obvious advantage of the 24-hour APC procedure over the official technique is that of time. Because the rapid procedure requires half the time, it would serve ideally as a check both along the line (as in the Hazard Analysis Critical Control Point Concept)

and on the final product before leaving the plant. It should be emphasized, however, that the rapid procedure, although statistically comparable to the official technique, consistently produced data indicating a lesser degree of recovery. Basically, the Eugon agar used in the procedure supplies the bacteria with a greater concentration of glucose, thus raising the metabolic rate and promoting hastened colony production. Although adequate, the nutritionally superior Eugon agar did not promote growth to the extent of the 48-hour incubation time with PCA in the official technique.

It was not the intent to recommend the 24-hour procedure be used in place of the official technique in determining the APC of frozen breaded shrimp. It is believed, however, that the rapid procedure is better suited in the food industry for routine quality control, process monitoring, and "red flag" situations.

Numbers of bacteria in frozen breaded shrimp have been reported by several investigators. Gunderson et al. (1954) in the first such survey reported a range of 9.5×10^5 to 14.0×10^6/g. Other surveys of frozen breaded shrimp have yielded 2.3×10^4/g to 54.0×10^6/g (Kachikian et al., 1959), 1.1×10^4 to 6.8×10^6/g (Vanderzant et al., 1973), and 2.4×10^4 to 60.0×10^6/g (Nickerson and Pollack, 1971). Such broad ranges were also noted in this interlaboratory investigation.

The treatment given in Table IV arranges the data from the 16 cooperating laboratories as data gathered from

independent APC analysis of frozen breaded shrimp using the official procedure (AOAC, 1970). With the exception of laboratories 1 and 16, all of the analytical laboratories reported an upper end greater than 1.0×10^6/g. These data were in agreement with previous reports. The lower end of the ranges reported were considerably less than reported in previous surveys of frozen raw breaded shrimp. Nine of the 16 laboratories reported lower limits of less than 1.0×10^4 bacteria/g. Four laboratories reported lower limits of less than 5000/g. In only one previous study dealing with cold-water shrimp off the coast of Maine were lower limits reported (Zapatka and Bartolomeo, 1973).

Table IV. APC Range and Number of Observations $>10^6$ Reported by 16 Analytical Laboratories

Laboratory Code Number	Range APC/g	Number of Observations	No. $>10^6$ APC/g
1	8,600-160,000	2	-
2	15,000-4,200,000	15	4
3	62,000-3,000,000	9	3
4	9,100-2,900,000	9	1
5	1,500-4,300,000	14	4
6	9,000-5,000,000	18	5
7	4,300-4,300,000	16	4
8	8,000-5,000,000	9	1
9	12,000-5,500,000	7	4
10	3,900-3,300,000	8	3
11	4,000-4,300,000	18	2
12	3,000-4,000,000	15	6
13	30,000-1,900,000	15	2
14	9,000-2,700,000	8	1
15	30,000-2,800,000	8	1
16	41,000-130,000	2	-
Overall	3,000-5,500,000	170	41

It is the consensus of most investigators that raw, frozen breaded shrimp produced under good manufacturing practices should contain less than 1,000,000 bacteria/g (Kachikian et al., 1959; Nickerson and Pollack, 1972; Silverman et al., 1973). Vanderzant et al. (1973) reported that of 276 samples of frozen breaded shrimp, 30% contained 1,000,000 bacteria/g or more. Nickerson and Pollack (1971) reported 48% of 136 samples contained 1,000,000 or more per gram as did Silverman et al. (1961) with 27% of 91 samples of breaded shrimp. Data gathered currently indicated that approximately 24% of 170 samples of frozen breaded shrimp produced APC values of 1,000,000 or more per gram.

Coliform Values for Frozen Raw Breaded Shrimp

In order to facilitate the statistical analysis of the data reported to the LSU Department of Food Science, the Most Probable Number (MPN) codes reported as 0-0-0 were assigned the value of 0.5 coliforms/g.

In Table V are listed the individual mean values, arranged in descending order of magnitude, of the 10 different sources of frozen raw breaded shrimp. The number of observations for total coliforms/source are also given.

The mean coliform values for the 10 different sources of breaded shrimp ranged from 9.3 MPN/g to 725.9 MPN/g. This range corresponded to an approximate 78-fold difference in extremes.

Table V. Mean Coliform MPN Values for 10 Different Sources of Frozen Raw Breaded Shrimp Reported by 16 Analytical Laboratories

Rank	Source Code Number	Observations per Source	Mean Coliform (MPN/g)
1	9	14	725.9
2	6	51	395.6
3	5	40	259.4
4	2	36	93.1
5	8	18	75.8
6	1	18	65.2
7	10	21	48.7
8	3	69	28.2
9	7	32	9.6
10	4	40	9.3

The coliform MPN mean values reported for sources 5, 6, and 9 were noticeably higher than the other sources. The mean values reported for sources 4 and 7 were, however, extremely low when compared to the other laboratories. In addition, the coliform MPN mean values for the remaining sources were comparable. The mean for the three highest values, sources 5, 6, and 9, was 460.3 coliforms/g. The mean of the lower levels, sources 9 and 10, was 9.5. There was an approximate 48-fold difference between the extremes. The difference in the two values demonstrated the variation in MPN coliform values for breaded shrimp from different processors.

The finding of a high variation between sources was not unexpected and was in agreement with results for the APC of frozen raw breaded shrimp. As with the APC of the samples, the variation in the coliform MPN may be due to varying

harvesting areas, ambient temperatures, and processing areas.

The mean coliform observations associated with the 16 participating alaytical laboratories, the number of observations per laboratory, and the overall values are listed in Table VI.

Table VI. Mean Coliform MPN Values for Frozen Raw Breaded Shrimp Reported by 16 Analytical Laboratories

Laboratory Code Number	Observations per Laboratory	Mean Coliform (MPN/g)
1	4	175.2
2	28	66.9
3	16	137.7
4	18	249.9
5	29	73.8
6	36	71.8
7	37	247.1
8	18	103.3
9	12	199.6
10	17	140.2
11	36	173.3
12	27	100.9
13	31	128.1
14	10	5.0
15	16	327.4
16	4	561.5
Overall	168	173.1

The F value for laboratory was 1.96 which was significant ($p < 0.05$); the critical values for F at 15/289 were $F_{.05} = 1.67$ and $F_{.01} = 2.04$. The observations for coliforms reported by the analytical laboratories ranged from an MPN of 5.0/g to 561.5/g. The low end of the range reported by laboratory 10 was noticeably low and undoubtedly contributed to the significant value of F.

There are no reports in the literature concerning the

reproducibility of the MPN coliform procedure in the routine bacteriological examination of seafoods. However, the data indicated that a very broad variation occurs in the analysis for coliforms between laboratories, but not necessarily within the same laboratory.

Table VII. Mean Coliform Values and Number of Observations Reported by 16 Analytical Laboratories Using the Official and Rapid Techniques

Laboratory Code Number	Official Technique		Rapid Technique	
	Observations per Laboratory	Mean Coliform (MPN/g)	Observations per Laboratory	Mean Coliform (MPN/g)
1	2	230.2	2	120.2
2	12	35.9	16	90.2
3	8	177.2	8	98.2
4	9	248.9	9	250.8
5	14	101.0	15	48.5
6	18	79.2	18	64.4
7	18	309.5	19	187.9
8	9	150.6	9	56.0
9	6	199.6	6	199.6
10	9	133.8	8	147.6
11	18	154.8	18	191.8
12	15	99.9	12	102.2
13	15	159.8	16	98.4
14	5	5.0	5	5.0
15	8	319.2	8	335.7
16	2	561.5	2	561.5
Overall	168	185.4	171	159.8

The mean coliform MPN values associated with the official method (AOAC, 1970) and the rapid technique are listed in Table VII. The F value for the interaction, technique x laboratory was 0.21 which was not significant ($p > 0.05$); the critical values for F at d.f. 15/289 were $F_{.05} = 1.67$ and $F_{.01} = 2.04$. This indicated that with respect to the mean

coliform MPN of the raw frozen breaded shrimp, the relationship between laboratories and technique was maintained throughout the analyses of all samples.

The F value for technique was 0.32 which was not significant ($p > 0.05$); the critical values for F at d.f. 1/289 were $F_{.05} = 3.84$ and $F_{.01} = 6.63$. The overall average of 159.8/g for the rapid procedure was smaller than the average of 185.4/g for the official technique. The means for the individual laboratories did not indicate the trend for higher values using the official procedure as readily as that for the APC, however.

Data gathered for the mean coliform MPN values for the two techniques were greater for the rapid techniques from analytical laboratories 2, 4, 9, 11, 12, and 15, while equal values were reported by laboratories 9, 14, and 16. Laboratories 1, 3, 5, 7, and 8 reported data which indicated the official procedure yielded appreciatively greater recovery of coliforms than did the rapid procedure.

Finding no significant difference ($p > 0.05$) in the rapid technique compared with the official procedure for coliforms in conjunction with the constancy of the relationship of the various laboratories indicated certain advantages for the rapid procedure. The latter, as well as the official technique, although resulting in broad variation between laboratories, produced consistent data in the same laboratory. Accepting this and the additional absence of a statistical difference ($p > 0.05$) between the procedures, the

obvious advantage of 24 hours instead of 96 hours becomes more attractive. The 3-day difference would prove to be a valuable asset in the application of quality control, process monitoring, and "red flag" situations.

It should be emphasized, however, that the rapid coliform MPN procedure is not intended to substitute for the official procedure. The latter is more expensive and requires additional steps which bring about the need for more time. These steps, however, are procedures which eliminate inherent errors in the MPN procedures. The official method is used in the study as a standard of comparison.

Numbers of coliforms in raw frozen breaded shrimp have been studied by several investigators. Kachikian et al. (1959) reported that of 144 samples of shrimp, 98 or 68% contained coliforms, with a range of 0 to 700 coliforms/g of sample. Gunderson et al. (1954) indicated a range of 150 to 1.4×10^4/g in 27 samples and Nickerson and Pollack (1972) a range of 0 to 8.9×10^3 coliforms/g in 136 samples.

Surkiewicz et al. (1967), in reporting data obtained from a 3-year study, suggested that frozen breaded raw shrimp produced under good manufacturing practices should have less than 1000 coliforms/g. This was supported in a later study (Vanderzant et al., 1973) where 276 processing plant samples were investigated; in the latter study the coliform numbers ranged from 3.6 to 4.6×10^3/g and only 8% contained more than 1000 coliforms/g.

Broad ranges for numbers of coliforms in breaded

shrimp were also indicated from the data in this study. Table VIII arranges the data obtained from the 16 analytical laboratories that reported the numbers of coliforms/g of breaded shrimp shipped to them. The results listed were obtained by the official procedure (AOAC, 1970). MPN values given as 0 were taken from the tube-MPN code of 0-0-0.

Table VIII. Coliform MPN Range and Number of Observations $>10^3$/g Reported by 16 Analytical Laboratories

Laboratory Code Number	Range MPN/g	Number of Observations	No. $>10^3$ MPN/g
1	*0-460	2	-
2	0-276	12	-
3	0-1100	8	1
4	0-1100	9	2
5	0-780	14	-
6	0-910	18	-
7	0-1100	18	3
8	0-1100	9	2
9	0-1100	6	1
10	0-1100	9	1
11	0-1100	18	1
12	0-680	15	-
13	0-237	15	-
14	0-12	5	-
15	0-460	8	-
16	23-1100	2	1
Overall	0-1100	168	14

*0 MPN code reported as 0-0-0.

Data indicated that 14 of 168 (8%) of the samples of frozen breaded shrimp tested by the official procedure contained more than 1000 coliform/g. This figure was in close agreement with the report of Vanderzant et al. (1973) who reported that 8% of 276 samples of raw frozen breaded shrimp contained more than 1000 coliforms/g. The data in this

study also supported the suggestion of Surkiewicz et al. (1967) that raw frozen breaded shrimp can easily be produced with less than 1000 coliforms/g. It should also be noted that over 22% of the samples tested by the AOAC technique were negative for coliforms. In addition to the absence of coliforms in a large proportion of the total sample, 143 or approximately 85% of the 270 samples contained less than 300 coliforms/g. Overall, only 15% of the 270 samples contained an MPN value of 460/g or more.

Coliforms have been reported by several investigators as not a member of the bacteriological flora in and on fresh shrimp (Williams and Rees, 1952b; Williams et al., 1952a; Harrison and Lee, 1969; Cobb et al., 1973). Williams et al. (1952c) and Williams and Rees (1952b) demonstrated the absence of coliforms in fresh shrimp from the Gulf of Mexico. They suggested the volume of their samples to be such that conclusions could be drawn, thus it was their opinion that coliforms of any type are not normally present on the raw product. Taking these reports into consideration, and the pattern of percentages which was indicated by the data in this study, the level of 1000 coliforms/g in the final breaded product imposed by the study of Surkiewicz et al. (1967) as the borderline of sanitary measurement seemed lenient.

Escherichia coli in Frozen Raw Breaded Shrimp

As with coliforms, raw frozen breaded samples reported as having an MPN code of 0-0-0 or no E. coli/g were given the value of 0.5 E. coli/g to facilitate statistical analysis.

The 10 source mean MPN E. coli values ranged from 0.5/g to 17.1 E. coli/g. In Table IX are listed the various MPN mean values for the 10 sources of raw frozen breaded shrimp, arranged in descending order of magnitude together with the number of observations reported by the laboratories.

Table IX. Mean E. coli MPN Values for the 10 Sources of Frozen Raw Breaded Shrimp

Rank	Source Code Number	Observations per Source	Mean E. coli (MPN/g)
1	1	18	17.1
2	9	14	7.6
3	5	40	1.6
4	2	38	1.2
5	6	52	1.1
5	3	69	1.1
6	7	33	1.0
7	4	39	0.6
8	8	16	0.5
8	10	20	0.5

A finding of a large difference between the replicates was not unexpected and was consistent with the quality tests for APC and total coliforms for the frozen breaded shrimp in this study. It is of interest that the E. coli

MPN source noted as code 1 was notably higher than the remaining nine. This obviously influenced the balance of data, affecting the value of F.

Sources coded 2-8 and 10 produced shrimp as reported by the analytical laboratories, containing comparable MPN levels of E. coli.

The mean values associated with the 16 laboratories ranged from 0.5 MPN/g to 9.8 E. coli MPN/g. The mean MPN E. coli reported by the laboratories was 1.85/g. The mean values computed for the individual laboratories are listed in Table X.

Table X. Mean E. coli MPN Values for Frozen Raw Breaded Shrimp Reported by 16 Analytical Laboratories

Laboratory Code Number	Observations per Laboratory	Mean E. coli (MPN/g)
1	4	0.5
2	25	9.8
3	16	0.5
4	19	1.6
5	28	1.0
6	37	3.6
7	36	0.8
8	18	1.4
9	12	0.5
10	17	1.8
11	37	1.9
12	30	1.7
13	30	1.5
14	10	0.5
15	16	1.3
16	4	1.2
Overall	339	1.85

The F value for laboratories was 1.59 which was not significant ($p > 0.05$); the critical values for F at 15/289 were $F_{.05} = 1.67$ and $F_{.01} = 2.04$. No reports in the literature investigated the reproducibility of the E. coli MPN technique in seafoods. Data presented in this study indicated that there were no significant differences between the laboratories analyzing series of identical shrimp samples.

The mean E. coli values reported by the participating laboratories associated with the official (AOAC, 1970) and rapid techniques are listed in Table XI. The F value for technique was 0.47 which was not significant ($p > 0.05$); the critical values for F at 1/289 were $F_{.05} = 3.84$ and $F_{.01} = 6.63$. In 3 of the analytical laboratories--1, 3, and 9--the mean E. coli MPN obtained by both the rapid and official techniques were identical. The rapid procedure produced greater means as reported by laboratories 2, 11, 12, and 13. Laboratories 4, 5, 6, 7, and 8 reported means greater for the official technique. The overall mean for the rapid procedure, unlike the APC and total coliform techniques, was greater than that for the official technique.

The F value for the interaction, technique x laboratory was 0.71 which was not significant ($p > 0.05$). The critical values for F at 15/289 were $F_{.05} = 1.67$ and $F_{.01} = 2.04$. This finding indicated that with respect to the mean E. coli MPN values for frozen raw breaded shrimp, the relationship between laboratories was consistently maintained for all samples.

Table XI. Mean E. coli MPN Values and Number of Observations Reported by the Analytical Laboratories Using the Official and Rapid Techniques

Laboratory Code Number	Official Technique		Rapid Technique	
	Observations per Laboratory	Mean E. coli (MPN/g)	Observations per Laboratory	Mean E. coli (MPN/g)
1	2	0.5	2	0.5
2	13	4.8	12	15.3
3	8	0.5	8	0.5
4	10	2.7	9	0.5
5	14	1.5	14	0.5
6	18	0.9	19	2.5
7	18	0.9	18	0.6
8	9	1.8	9	1.1
9	6	0.5	6	0.5
10	9	3.0	8	0.5
11	19	0.6	18	3.3
12	15	1.2	15	2.2
13	15	1.4	15	1.5
14	5	0.5	5	0.5
15	8	0.8	8	1.9
16	2	2.0	2	0.5
Overall	171	2.0	168	1.7

The rapid technique for the enumeration of E. coli investigated was identical to that suggested by Fishbein et al. (1967) and adopted by official agencies (U.S. Dept. of Health, Education, and Welfare, 1969) as a rapid but not standard procedure. The technique was not as lengthy as the standard procedure, there being a 4-day difference in time required for obtaining final results. The longer procedure, however, was more exacting, requiring primary and secondary MPN procedures as well as final confirmation on a differential medium.

The numbers of E. coli in frozen raw breaded shrimp have been reported by several investigators. Vanderzant et.

al. (1973) reported that of 276 samples, E. coli was recovered from 8% and at very low levels. Nickerson and Pollack (1971) reported that 20% of the samples tested over a 3-year period were positive for E. coli.

Data listed in Table XII are arranged as E. coli MPN values from this interlaboratory study as independent breaded shrimp samples. The range reported by the participating laboratories, the number of observations by the laboratory, and the percentage of those observations positive are listed.

Table XII. MPN Range, Number of Observations, and Percent of Frozen Breaded Shrimp Samples Positive for Escherichia coli

Laboratory Code Number	Range MPN/g	Number of Observations	Positive	
			Number	Percent
1	-	2	0	-
2	*0-60	13	5	42
3	-	8	0	-
4	0-23	10	1	11
5	0-5.5	14	5	36
6	0-36	18	3	17
7	0-6.1	18	3	17
8	0-6.3	9	2	22
9	-	6	0	-
10	0-33	9	1	11
11	0-3	19	1	6
12	0-9.1	15	1	7
13	0-11	15	4	28
14	-	5	0	-
15	0-3	8	1	13
16	0-3.6	2	1	50
Overall	0-60	171	28	16.3

*0 MPN code reported as 0-0-0.

The data indicated broad variations between the laboratories when compared individually. The percentage of the samples from which E. coli was recovered varied from 6 to 50%, the latter based on only 2 samples. The overall percentage using 171 samples appeared to be in agreement with the literature. These data indicated that 16% of the samples were positive which compared well with the range of values published by Vanderzant et al. (1973), Surkiewicz et al. (1967), and Nickerson and Pollack (1971), who indicated 8%, 20%, and 34%, respectively.

Coagulase-Positive Staphylococci Values for Breaded Shrimp

The mean coagulase-positive staphylococci values for the 10 sources of frozen breaded shrimp ranged from an MPN of 9.2/g to 261.0/g. In Table XIII are listed the individual source mean values, arranged in descending order of magnitude, for the 10 sources of raw frozen breaded shrimp.

Table XIII. Mean Coagulase-Positive Staphylococci MPN Values for the 10 Sources of Raw Frozen Breaded Shrimp

Rank	Source Code Number	Observations per Source	Mean (MPN/g)
1	10	20	261.0
2	8	17	152.4
3	3	69	108.7
4	4	37	63.5
5	6	53	49.9
6	7	34	27.2
7	2	33	19.2
8	1	18	16.7
9	5	42	9.2
10	9	14	7.7

The finding of broad differences between sources for coagulase-positive staphylococci MPN was in agreement with APC, coliforms, and E. coli. Essentially the data indicated that shrimp produced in varying sections of the United States differ markedly in their bacteriological characteristics.

The mean coagulase-positive staphylococci values associated with the 16 reporting laboratories ranged from an MPN of 2.6/g to 199.1/g, which corresponded to an approximate 76-fold difference in extremes. The mean coagulase-positive staphylococci MPN values along with the number of observations are listed in Table XIV.

Table XIV. Mean Coagulase-Positive Staphylococci MPN Values for Raw Frozen Breaded Shrimp Reported by 16 Analytical Laboratories

Laboratory Code Number	Observations per Laboratory	Mean (MPN/g)
1	4	2.6
2	23	137.2
3	16	25.8
4	18	199.1
5	30	47.2
6	36	87.8
7	36	89.6
8	19	24.5
9	12	96.1
10	18	30.6
11	36	45.8
12	30	15.3
13	30	7.7
14	12	75.1
15	15	150.3
16	2	15.8
Overall	337	65.6

The F value for laboratories was 1.74 which was significant ($p < 0.05$). The critical values for F at d.f. 15/287 were $F_{.05} = 1.67$ and $F_{.01} = 2.04$. This was not in agreement with the other quality tests investigated. Essentially, there was a large variation in data between laboratories in regard to testing for coagulase-positive staphylococci in frozen breaded shrimp.

The mean coagulase-positive staphylococci values associated with the official (AOAC, 1970) and the rapid techniques are listed in Table XV.

Table XV. Mean Coagulase-Positive Staphylococci MPN Values and Number of Observations Reported by 16 Analytical Laboratories Using the Official and Rapid Techniques

Laboratory Code Number	Official Technique		Rapid Technique	
	Observations per Laboratory	Mean (MPN/g)	Observations per Laboratory	Mean (MPN/g)
1	2	0.5	2	4.8
2	11	39.4	12	226.8
3	8	6.8	8	44.8
4	9	310.7	9	87.5
5	15	7.1	15	87.3
6	18	17.4	18	158.3
7	18	89.3	18	89.8
8	9	35.8	10	14.3
9	6	5.6	6	186.6
10	9	4.0	9	57.3
11	18	1.4	18	90.1
12	15	9.0	15	21.7
13	15	1.8	15	13.6
14	6	55.4	6	94.9
15	7	159.2	8	142.5
16	1	28.0	1	3.6
Overall	167	48.2	170	54.5

The F value for technique was 1.96 which was significant ($p < 0.05$); the critical values for F at d.f. 1/287 were $F_{.05} = 3.84$ and $F_{.01} = 6.63$. The finding of a significant difference between techniques was not in agreement with the other quality tests. Only in laboratories 7 and 15 were the values for the official and rapid tests comparable. The extremes of the range for the official coagulase-positive staphylococci, 0.5 to 310.7/g, represented an approximate 621-fold difference. The rapid procedure yielded a 47-fold difference between extremes. Twelve of the 16 participating laboratories reported greater values for the rapid procedure, a trend opposite from the APC and also contrary to coliforms and E. coli MPNs which did not set a definite trend.

The broad variation in the result of both the official and rapid techniques are believed to be due to the complexity of the techniques and/or the dependence of individual judgment by the technician. The official technique requires a minimum of 5 days and has in it several steps which are critical in effecting reproducible results. The addition of potassium tellurite at the proper temperature was thought to be a critical step in preparing the differential medium used in the secondary MPN estimation. Another problem area was the actual reading of the coagulase test itself, which though simple when strongly positive, becomes increasingly more difficult as the positivity of the strain under test lessens. Other factors which may have introduced error in the results of the official procedure were the selection of

colony types and the number selected, i.e., whether enough colonies were picked from the differential agar by the various technicians for the confirmatory, coagulase plasma MPN.

The rapid procedure, which was a simple determination of color change after 24 hours, was as variable as the official procedure. The rapid method was based on the selectivity of one medium, Tryptic Meat Mannitol broth, which has incorporated into it two selective agents, 10% NaCl and Polymyxin B. The differential agent was mannitol in conjunction with phenol red as a pH indicator. The test required noting only an MPN code after the incubation period based on color change due to the fermentation of mannitol. Close association between coagulase positivity and mannitol fermentation by Staphylococcus aureus has been reported by several authors (Gwatkin, 1937; Joshi and Dale, 1963; Zemelman and Longeri, 1965).

The 24-hour technique cannot replace the official 5-day method. The latter has incorporated in it additional steps which act as limiting factors for inherent errors in the procedure. These include secondary MPN procedures on differential media and confirmatory MPN procedures in coagulase plasma. These steps which may have apparently reduced recovery, as determined by the overall mean values in Table XV, more than likely increase the accuracy of the technique. The rapid method, however, is believed to be useful when industrial situations arise where quick results are necessary.

As a monitoring system, any fluctuation from the expected values would indicate potential problems. The 5-day method does not lend itself well to monitoring techniques, in-line testing, and in "red flag" situations.

The numbers of coagulase-positive staphylococci in frozen raw breaded shrimp has not been well documented as have been the other indicator organisms. The data provided by the literature survey were not consistent, but the differences may have resulted from the difficulty of the analytical procedures. Gunderson et al. (1954) reported that coagulase-positive Staphylococcus were present in all of the samples in their survey. Surkiewicz et al. (1967), in a broad 3-year study, reported data suggesting that 43% of the samples tested were positive for coagulase-positive staphylococci. Vanderzant et al. (1973) indicated that of 276 samples of frozen breaded shrimp, 50% contained coagulase-positive staphylococci. Other studies produced data indicating 75% of 91 samples (Silverman et al., 1962) and 10% of 121 samples of frozen raw breaded shrimp (Zapatka and Bartolomeo, 1972) were positive for coagulase-positive staphylococci. The latter investigation was restricted to cold-water shrimp harvested and processed in and around the Gulf of Maine.

The data in Table XVI are arranged as MPN coagulase-positive staphylococci values reported by the participating laboratories as independent results obtained by the official technique (AOAC, 1970). Also listed are the ranges for each

laboratory, number of observations per laboratory, and percentages positive for coagulase-positive staphylococci.

Table XVI. Coagulase-Positive Staphylococci MPN Ranges and Percent Positive in Raw Frozen Breaded Shrimp Reported by 16 Analytical Laboratories

Laboratory Code Number	Range MPN/g	Number of Observations	Percent Positive
1	*0-3.6	2	50.0
2	0-276	11	91.6
3	0-51.1	8	75.0
4	4.5-1100	9	100.0
5	0-43	15	50.0
6	0-91	18	33.3
7	3.6-1100	18	100.0
8	0-120	9	27.7
9	0-23	6	33.3
10	0-9.1	9	77.7
11	0-9.1	18	38.8
12	0-85	15	50.0
13	0-33	15	33.3
14	4.5-166.5	6	100.0
15	0-1100	8	50.0
16	15.5	1	100.0
Overall	0-1100	168	59.6

*0 MPN code reported as 0-0-0.

A broad variation in the reporting laboratories was noted in the samples positive for coagulase-positive staphylococci. However, the overall average of positive samples agreed closely to that reported in previous investigations (Surkiewicz et al., 1967; Vanderzant et al., 1973). The overall percentage of the samples with less than 100 coagulase-positive staphylococci/g was lower than expected. According to the study by Surkiewicz (1967), plants with good manufacturing practices produced raw frozen breaded

shrimp with no more than 1000 coagulase-positive staphylococci/g as a geometric average. These data indicated that this was a lenient figure since the percentage of samples positive for coagulase-positive staphylococci in numbers equal to or greater than 100/g was only slightly above 10%.

SUMMARY

The raw frozen seafood industry is uniquely susceptible to bacteriological problems. Their products undergo no bacteriocidal procedure other than the slow one related to the frozen state. Consequently, the bacteriological analysis of the raw product, in-line process monitoring, and finished product are of the utmost importance. Unfortunately, bacteriological tests used to assess quality require up to 4 days, the maximum duration allowed by the U.S. Food and Drug Administration. In that time, the frozen, breaded product has been distributed, placed on retail shelves, and could easily have been consumed. More rapid tests which can be used to monitor the product and show the bacterial quality before it leaves the plant are needed.

Such rapid procedures were developed for the determination of the aerobic plate count (APC), coliforms, E. coli, and coagulase-positive staphylococci in raw frozen breaded shrimp. All techniques required 24 hours for completion.

The data compiled in this study were produced as part of a cooperative effort involving 16 analytical laboratories. The data were used to determine the feasibility of the rapid techniques by comparing those results to the official methods. Analysis of the data indicated the following: 1) The rapid procedures designed to facilitate monitoring of APC, total

coliforms, and E. coli produced data not significantly different ($p > 0.05$) from the official methods. 2) The bivariate interaction, laboratory x technique, was not significant ($p > 0.05$) for APC, total coliforms, and E. coli.

These results support the proposed rapid procedures for APC, coliforms, and E. coli. In addition, since the interaction, technique x laboratory, is not significant, the relationship between the official and rapid procedure was maintained for each test with respect to the mean value.

The rapid test for coagulase-positive staphylococci was significantly different ($p < 0.05$) from the official method. The results given by both techniques were consistent as the bivariate interaction, technique x laboratories, was not significant ($p > 0.05$). The broad variations in the means reported by the laboratories were accredited to the relative difficulty of the official method and independent bias of the technicians involved.

The rapid procedures tested are considerably more feasible in the frozen food industry for quality evaluation than the timely official methods. The rapid methods allow assessment of the complete bacteriological profile of the product before it is at the retail level. The procedures, when used continuously, also would indicate abnormal changes in the bacterial characteristics of the product 72 hours before official methods.

Analysis of the data on an interlaboratory basis indicated a significant different ($p < 0.05$) between means for

total coliforms and coagulase-positive staphylococci. The bivariate interaction, laboratory x technique, however, was not significant ($p > 0.05$). This indicated that the laboratories maintained the relationship between the official and newly developed, rapid techniques.

The APC range for all samples of frozen breaded shrimp was from 3000/g to 5.5×10^6/g with 25% containing 1.0×10^6 bacteria/g or more. Coliforms were found in 78% of the samples with a range of 23/g to 1100/g using the official MPN method. Eight percent of the samples yielded coliform MPN values of 1000/g or more, while 85% yielded MPN values of less than 300 coliforms/g. E. coli was recovered from 16% of the frozen breaded shrimp samples with an MPN range of 0 to 60/g. The MPN range for coagulase-positive staphylococci was 0 to 1100/g. The majority of the samples, 59.6%, was positive with only 10% of those containing an MPN of 100/g or more.

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APPENDIX

Table A. Raw Data from Interlaboratory Bacteriological Analyses of Frozen Raw Breaded Shrimp, AOAC Results

SM	AOAC Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus (MPN/g)
Laboratory 1:				
309	8.2	0.5	0.5	0.5
	9.0	0.5	0.5	3.6
916	160.0	460.0	0.5	0.5
Laboratory 2:				
201	25.0	43.0	3.0	3.6
	26.0	93.0	0.5	3.6
502	780.0	93.0	3.0	0.5
	1100.0	460.0	0.5	0.5
403	15.0	3.0	0.5	23.0
	20.0	3.0	0.5	93.0
104	1700.0	43.0	43.0	3.6
	1800.0	75.0	75.0	0.5
305	62.0	23.0	3.6	43.0
	56.0	15.0	0.5	9.1
606	1200.0	43.0	3.0	240.0
	1000.0	240.0	0.5	43.0
707	690.0	43.0	0.5	15.0
	1100.0	0.5	0.5	23.0
808	200.0	0.5	0.5	240.0
	170.0	0.5	0.5	93.0
309	17.0	9.1	0.5	23.0
	13.0	23.0	0.5	9.1
1010	4200.0	93.0	0.5	3.6
	4300.0	93.0	0.5	3.6
311	1100.0	23.0	0.5	43.0
	1200.0	0.5	0.5	23.0
513	4.3	23.0	0.5	23.0
Laboratory 3:				
201	66.0	93.0	0.5	0.5
	140.0	43.0	0.5	9.1
502	2400.0	1100.0	0.5	0.5
	1700.0	1100.0	0.5	0.5

Table A (continued)

SM	AOAC Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus (MPN/g)
104	73.0	0.5	0.5	3.0
305	43.0	23.0	0.5	9.1
	280.0	23.0	0.5	93.0
808	130.0	93.0	0.5	9.1
309	62.0	0.5	0.5	0.5
1010	3000.0	93.0	0.5	9.1
311	970.0	15.0	0.5	23.0
Laboratory 4:				
309	100.0	23.0	23.0	1100.0
1010	210.0	3.6	0.5	460.0
311	2900.0	0.5	0.5	1100.0
412	9.2	3.6	0.5	9.1
	9.0	0.5	0.5	0.5
513	43.0	0.5	0.5	9.1
	74.0	0.5	0.5	9.1
715	19.0	0.5	0.5	9.1
916	190.0	1100.0	0.5	23.0
	250.0	1100.0	0.5	21.0
217	45.0	9.1	0.5	43.0
	52.0	9.1	0.5	93.0
618	99.0	1100.0	0.5	43.0
	174.0	1100.0	0.5	23.0
Laboratory 5:				
201	110.0	93.0	0.5	3.6
	190.0	460.0	0.5	3.0
502	2500.0	1100.0	0.5	0.5
	2500.0	460.0	0.5	0.5
403	35.0	3.6	0.5	43.0
	30.0	3.6	0.5	3.6
305	64.0	75.0	3.6	0.5
	71.0	93.0	7.3	9.1
606	2200.0	460.0	3.6	9.1
	1800.0	460.0	3.6	3.6

Table A (continued)

SM	AOAC Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus MPN/g)
707	140.0	23.0	0.5	43.0
	190.0	9.1	3.6	43.0
808	52.0	39.0	0.5	0.5
	48.0	23.0	0.5	23.0
1010	4200.0	240.0	0.5	0.5
	4400.0	1100.0	0.5	0.5
311	1300.0	6.2	6.2	3.6
	1300.0	0.5	0.5	0.5
412	1.0	9.1	3.6	0.5
	2.0	9.1	3.6	0.5
513	66.0	0.5	0.5	0.5
	84.0	3.6	0.5	0.5
614	83.0	3.6	0.5	0.5
	88.0	3.6	0.5	0.5
715	73.0	0.5	0.5	0.5
	62.0	0.5	0.5	0.5
Laboratory 6:				
201	1600.0	91.0	0.5	0.5
	1700.0	91.0	0.5	0.5
502	5000.0	93.0	9.0	23.0
403	93.0	36.0	0.5	91.0
	750.0	36.0	0.5	0.5
104	1100.0	36.0	36.0	0.5
	1300.0	36.0	36.0	0.5
305	53.0	91.0	0.5	91.0
606	753.0	0.5	0.5	0.5
707	430.0	36.0	0.5	91.0
808	27.0	36.0	0.5	0.5
	31.0	0.5	0.5	0.5
309	96.0	3.6	0.5	0.5
	89.0	0.5	0.5	0.5
1010	1900.0	0.5	0.5	0.5
311	1200.0	3.0	0.5	0.5
	1200.0	0.5	0.5	0.5

Table A (continued)

SM	AOAC Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus (MPN/g)
412	9.0	0.5	0.5	9.0
513	74.0	0.5	0.5	0.5
614	60.0	0.5	0.5	0.5
715	80.0	0.5	0.5	3.6
916	300.0	910.0	0.5	0.5
	170.0	910.0	36.0	0.5
217	110.0	0.5	0.5	0.5
	108.0	0.5	0.5	0.5
618	47.0	91.0	0.5	0.5
	34.0	0.5	0.5	0.5
Laboratory 7:				
201		1100.0	0.5	3.6
		43.0	0.5	21.0
502		1100.0	0.5	150.0
		1100.0	0.5	1100.0
403	135.0	23.0	0.5	1100.0
	87.0	3.6	0.5	23.0
104	3800.0	460.0	3.6	3.6
606	730.0	0.5	0.5	9.1
707	570.0	23.0	0.5	9.1
808	24.0	93.0	0.5	23.0
309	42.0	9.1	3.6	23.0
1010	2500.0	15.0	0.5	210.0
311	4300.0	23.0	0.5	3.6
412	4.3	23.0	0.5	91.0
513	50.0	23.0	0.5	3.6
614	69.0	9.1	0.5	9.1
715	77.0	9.1	0.5	9.1
916	380.0	1100.0	0.5	0.5
	420.0	960.0	0.5	3.6
217	68.0	9.1	0.5	3.6
618	1200.0	1100.0	0.5	3.6
	1000.0	1100.0	0.5	23.0

Table A (continued)

SM	AOAC Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus (MPN/g)
Laboratory 8:				
201	200.0 55.0	93.0 15.0	3.6 9.1	23.0 0.5
502	230.0 5000.0	93.0 1100.0	0.5 0.5	11.0 240.0
403	60.0 48.0	23.0 3.6	0.5 0.5	43.0 9.0
104	8.0 30.0	15.0 39.0	9.1 0.5	0.5 0.5
309	8.0	3.6	0.5	0.5
311	220.0 400.0	0.5 0.5	0.5 0.5	240.0 0.5
614	60.0	0.5	0.5	0.5
217	95.0 150.0	3.6 3.6	0.5 0.5	0.5 0.5
618	15.0 15.0	1100.0 1100.0	0.5 0.5	3.6 3.6
Laboratory 9:				
201	470.0 160.0	43.0 93.0	0.5 0.5	9.1 0.5
502	1300.0	1100.0	0.5	0.5
403	12.0	0.5	0.5	0.5
104	2300.0 2000.0			
309	14.0	0.5	0.5	0.5
1010	1900.0	3.6	0.5	0.5
311	5500.0	0.5	0.5	0.5
Laboratory 10:				
104	3600.0 3100.0	43.0 460.0	23.0 43.0	3.6 3.6
309	22.0	3.6	0.5	3.6
1010	2400.0	15.0	0.5	3.0
311	1100.0 930.0	0.5 0.5	0.5 0.5	0.5 0.5

Table A (continued)

SM	AOAC Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus (MPN/g)
412	3.0	23.0	0.5	9.1
513	62.0	3.6	0.5	3.6
614	57.0	15.0	0.5	0.5
715		0.5	0.5	3.6
618	20.0	1100.0	0.5	9.1
Laboratory 11:				
201	26.0	23.0	0.5	0.5
	24.0	15.0	0.5	0.5
502	1200.0	1100.0	0.5	0.5
	22.0	9.1	0.5	0.5
403	21.0	3.6	0.5	3.0
	34.0	0.5	0.5	3.0
104	17.0	0.5	0.5	0.5
305	37.0	43.0	0.5	0.5
606	530.0	14.0	0.5	0.5
707	52.0	3.6	3.0	0.5
808	12.0	0.5	0.5	0.5
309	78.0	0.5	0.5	0.5
1010	4300.0	0.5	0.5	3.6
311	1700.0	0.5	0.5	3.6
412	4.0	23.0	0.5	0.5
513	63.0	0.5	0.5	3.0
614	95.0	3.6	0.5	3.6
715	410.0	0.5	0.5	0.5
916	170.0	460.0	0.5	0.5
	320.0	460.0	0.5	0.5
217	160.0	3.6	0.5	3.6
	140.0	9.1	0.5	3.6
618	190.0	1100.0	0.5	0.5
	200.0	1100.0	0.5	9.1

Table A (continued)

SM	AOAC Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus (MPN/g)
Laboratory 12:				
201	1300.0	43.0	0.5	0.5
	950.0	23.0	0.5	0.5
502	900.0	9.1	0.5	0.5
	1100.0	23.0	0.5	0.5
403	30.0	7.3	0.5	
	30.0	0.5	0.5	
305	64.0	11.0	0.5	23.0
606	1200.0	9.1	0.5	3.6
707	1400.0	9.1	0.5	4.3
309	49.0	3.6	0.5	0.5
1010	3300.0	23.0	0.5	0.5
	4700.0	23.0	0.5	0.5
311	830.0	0.5	0.5	0.5
412	3.0	3.6	0.5	3.6
	160.0	3.6	0.5	3.0
614	93.0	7.3	0.5	0.5
715	1800.0	9.1	9.1	0.5
916	140.0	240.0	0.5	20.0
	290.0	460.0	0.5	150.0
217	100.0	23.0	0.5	43.0
	90.0	9.1	0.5	43.0
618	85.0	1100.0	0.5	23.0
	78.0	290.0	0.5	28.0
Laboratory 13:				
201	480.0	460.0	6.1	23.0
	62.0	15.0	0.5	0.5
502	740.0	460.0	3.0	0.5
	71.0	12.0	0.5	0.5
403	30.0	7.3	0.5	3.6
	30.0	0.5	0.5	0.5
104	30.0	0.5	0.5	0.5
	30.0	0.5	0.5	0.5
305	30.0	43.0	0.5	0.5
	41.0	240.0	11.0	0.5

Table A (continued)

SM	AOAC Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus (MPN/g)
606	640.0	240.0	7.2	0.5
	820.0	3.6	0.5	0.5
707	30.0	0.5	0.5	9.1
	30.0	3.6	0.5	9.1
808	39.0	0.5	0.5	0.5
	32.0	9.1	0.5	0.5
309	36.0	43.0	0.5	0.5
311	1900.0	23.0	0.5	23.0
	1600.0	43.0	0.5	43.0
412	30.0	0.5	0.5	0.5
	30.0	3.6	0.5	3.6
513	30.0	0.5	0.5	0.5
	41.0	0.5	0.5	0.5
614	30.0	9.1	0.5	0.5
	30.0	9.1	0.5	0.5
715	540.0	9.1	0.5	9.1
	630.0	9.1	0.5	9.1
618	1400.0	1100.0	0.5	0.5
	2400.0	1100.0	0.5	0.5
Laboratory 14:				
311	2900.0	0.5	0.5	240.0
	2600.0	3.6	0.5	93.0
412	9.0	23.0	0.5	23.0
	42.0	0.5	0.5	0.5
513	62.0	0.5	0.5	0.5
	4.0	0.5	0.5	9.1
614	1200.0	0.5	0.5	23.0
	38.0	0.5	0.5	23.0
715	540.0	0.5	0.5	23.0
Laboratory 15:				
808	2800.0	460.0	0.5	1100.0
309	81.0	23.0	0.5	9.1
412	31.0	3.6	0.5	11.0
	30.0	0.5	0.5	3.6

Table A (continued)

SM	AOAC Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus (MPN/g)
513	1200.0	0.5	0.5	3.6
	47.0	0.5	0.5	0.5
715	350.0	3.0	3.0	0.5
916	320.0	960.0	0.5	0.5
217	440.0	7.3	0.5	0.5
	420.0	3.6	0.5	0.5
618	220.0	1100.0	0.5	0.5
Laboratory 16:				
217	22.0	23.0	3.6	
	60.0	23.0	3.6	
618	120.0	1100.0	0.5	28.0
	140.0	1100.0	0.5	3.0

Table B. Raw Data from Interlaboratory Bacteriological Analyses of Frozen Raw Breaded Shrimp, Rapid Results

SM	Rapid Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus (MPN/g)
Laboratory 1:				
309	6.1	0.5	0.5	0.5
	7.3	0.5	0.5	3.6
916	99.0	240.0	0.5	9.1
Laboratory 2:				
201		43.0	3.6	23.0
		150.0	0.5	23.0
502		1100.0	23.0	23.0
		1100.0	23.0	23.0
403	16.0	3.0	0.5	19.0
	22.0	3.0	0.5	43.0
104	1900.0	43.0	150.0	0.5
	1900.0	75.0	120.0	0.5
305	45.0	23.0	0.5	75.0
	49.0	15.0	0.5	93.0
606	1100.0	43.0	3.0	1100.0
	940.0	93.0	0.5	240.0
707	680.0	15.0	0.5	75.0
	800.0	0.5	0.5	93.0
808	17.0	3.6	0.5	240.0
	210.0	3.0	0.5	93.0
309	12.0	9.1	0.5	23.0
	12.0	23.0	0.5	9.1
1010	4300.0	15.0	0.5	1100.0
	4600.0	93.0	3.6	210.0
311	1100.0	15.0	0.5	43.0
	1200.0	0.5	0.5	240.0
513	4.3	23.0	0.5	0.5
Laboratory 3:				
201	74.0	93.0	0.5	93.0
	152.0	53.0	0.5	240.0
502	2700.0	460.0	0.5	240.0
	2000.0	1100.0	0.5	0.5

Table B (continued)

SM	Rapid Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus (MPN/g)
104	84.0	0.5	0.5	23.0
305	36.0	23.0	0.5	0.3
	110.0	23.0	0.5	15.0
808	160.0	93.0	0.5	0.5
309	13.0	23.0	0.5	0.5
1010	3100.0	93.0	0.5	0.5
311	880.0	0.5	0.5	240.0
Laboratory 4:				
309	58.0	23.0	0.5	240.0
1010	87.0	15.0	0.5	460.0
311	2400.0	0.5	0.5	23.0
412	9.7	9.1	0.5	9.1
	3.0	0.5	0.5	0.5
513	26.0	0.5	0.5	0.5
	25.0	0.5	0.5	0.5
715	49.0	0.5	0.5	23.0
916	180.0	1100.0	0.5	9.1
	220.0	1100.0	0.5	3.0
217	60.0	9.1	0.5	23.0
	52.0	9.1	0.5	23.0
618	96.0	1100.0	0.5	0.5
	171.0	1100.0	0.5	0.5
Laboratory 5:				
201	63.0	43.0	0.5	93.0
	58.0	93.0	0.5	43.0
502	1100.0	240.0	0.5	43.0
	1300.0	240.0	0.5	78.0
403	26.0	3.6	0.5	23.0
	14.0	0.5	0.5	0.5
104	21.0	0.5	0.5	23.0
	18.0	0.5	0.5	93.0
305	31.0	20.0	0.5	150.0
	36.0	93.0	0.5	150.0

Table B (continued)

SM	Rapid Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus (MPN/g)
606	390.0	28.0	0.5	3.6
	430.0	9.3	0.5	23.0
707	66.0	23.0	0.5	3.0
	87.0	9.1	0.5	0.5
808	32.0	23.0	0.5	0.5
	37.0	23.0	0.5	0.5
1010	3500.0	240.0	0.5	240.0
	4000.0	240.0	0.5	240.0
311	1300.0	0.5	0.5	460.0
	1300.0	0.5	0.5	1100.0
412	1.0	9.1	0.5	0.5
	1.0	9.1	0.5	0.5
513	20.0	0.5	0.5	23.0
	22.0	3.6	0.5	7.2
614	63.0	3.6	0.5	240.0
	37.0	3.6	3.6	93.0
715	48.0	0.5	0.5	0.5
	42.0	0.5	0.5	0.5
Laboratory 6:				
201	1100.0	91.0	0.5	36.0
	1400.0	91.0	0.5	9.0
502	3000.0	9.0	3.6	0.5
403	95.0	0.5	0.5	360.0
	980.0	0.5	0.5	91.0
104	930.0	36.0	36.0	230.0
	1000.0	36.0	91.0	230.0
305	58.0	36.0	0.5	360.0
606	710.0	0.5	0.5	360.0
707	360.0	36.0	0.5	360.0
808	11.0	36.0	0.5	0.5
	12.0	0.5	0.5	0.5
309	39.0	0.5	0.5	36.0
	37.0	3.6	0.5	36.0
1010	1500.00	0.5	0.5	1100.0

Table B (continued)

SM	Rapid Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus (MPN/g)
311	1600.0	0.5	0.5	0.5
	1700.0	0.5	0.5	0.5
412	3.0	0.5	0.5	0.5
513	25.0	0.5	0.5	0.5
614	49.0	0.5	0.5	0.5
715	200.0	0.5	0.5	0.5
916	180.0	910.0	0.5	0.5
	170.0	910.0	0.5	0.5
217	120.0	0.5	0.5	3.0
	120.0	0.5	0.5	3.0
618	49.0	0.5	0.5	0.5
	23.0	0.5	0.5	0.5
Laboratory 7:				
201		460.0	0.5	78.0
		91.0	0.5	210.0
502		460.0	0.5	43.0
		460.0	0.5	23.0
403	110.0	23.0	0.5	460.0
	77.0	3.6	0.5	9.1
104	3000.0	460.0	0.5	3.6
305	90.0	460.0	3.6	23.0
606	710.0	0.5	0.5	210.0
707	510.0	23.0	0.5	23.0
808	14.0	23.0	0.5	91.0
309	38.0	9.1	0.5	91.0
1010	3000.0	3.6	0.5	460.0
311	1800.0	23.0	0.5	9.1
412	1.5	9.1	0.5	0.5
513	8.4	43.0	0.5	9.1
614	58.0	3.6	0.5	3.6
715	58.0	3.6	0.5	3.6
916	410.0	460.0	0.5	9.1
	320.0	460.0	0.5	23.0

Table B (continued)

SM	Rapid Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus (MPN/g)
217	47.0	3.6	0.5	9.1
618	800.0	1100.0	0.5	91.0
	950.0	1100.0	0.5	91.0
Laboratory 8:				
201	170.0	9.1	3.6	0.5
	40.0	9.1	0.5	0.5
502	250.0	21.0	0.5	0.5
	3000.0	150.0	0.5	0.5
403	50.0	3.6	0.5	0.5
	35.0	0.5	0.5	0.5
104	7.0	9.1	3.6	0.5
	31.0	9.1	0.5	0.5
309	4.0	0.5	0.5	0.5
311	300.0	0.5	0.5	93.0
	950.0	0.5	0.5	9.1
614	70.0	0.5	0.5	43.0
217	85.0	0.5	0.5	0.5
	100.0	0.5	0.5	0.5
618	10.0	460.0	0.5	3.6
	12.0	1100.0	0.5	9.1
Laboratory 9:				
201	230.0	93.0	0.5	15.0
	120.0	43.0	0.5	0.5
502	450.0	1100.0	0.5	0.5
403	6.0	0.5	0.5	0.5
104	1800.0			
	1400.0			
309	7.6	0.5	0.5	0.5
1010	1200.0	3.6	0.5	3.6
311	4600.0	0.5	0.5	1100.0

Table B (continued)

SM	Rapid Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus (MPN/g)
Laboratory 10:				
104	700.0	0.5	0.5	3.6
	280.0	0.5	0.5	0.5
309	8.7	3.6	0.5	23.0
1010	1900.0	15.0	0.5	23.0
311	790.0	0.5	0.5	460.0
	800.0	0.5	0.5	1100.0
412	3.0	43.0	0.5	0.5
513	13.0	9.1	0.5	0.5
614	45.0	9.1	0.5	0.5
715		0.5	0.5	0.5
618		1100.0	0.5	3.6
Laboratory 11:				
201	25.0	23.0	0.5	0.5
	10.0	93.0	0.5	0.5
502	1000.0	1100.0	0.5	0.5
	22.0	460.0	0.5	0.5
403	17.0	3.6	0.5	3.0
	9.0	0.5	0.5	3.0
104	29.0	0.5	0.5	0.5
305	20.0	23.0	0.5	0.5
606	540.0	14.0	0.5	0.5
707	15.0	23.0	0.5	0.5
808	14.0	0.5	0.5	0.5
309	50.0	21.0	0.5	0.5
1010	3400.0	7.3	0.5	1100.0
311	1200.0	0.5	0.5	460.0
412	3.0	23.0	0.5	9.1
513	33.0	0.5	0.5	3.6
614	85.0	3.6	0.5	23.0
715	230.0	0.5	0.5	3.6

Table B (continued)

SM	Rapid Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus (MPN/g)
916	130.0	1100.0	43.0	9.1
	140.0	460.0	0.5	23.0
217	110.0	9.1	0.5	7.3
	97.0	9.1	0.5	9.1
618	140.0	1100.0	9.1	0.5
	110.0	1100.0	0.5	0.5
Laboratory 12:				
201	900.0	23.0	0.5	0.5
	600.0	23.0	0.5	0.5
502	500.0	9.1	0.5	0.5
	600.0	23.0	0.5	0.5
403	30.0	0.5	0.5	9.1
	30.0	0.5	0.5	9.1
305	63.0		0.5	0.5
606	1100.0		0.5	7.3
707	1000.0		0.5	75.0
309	10.0	23.0	0.5	23.0
1010	4700.0	43.0	0.5	43.0
	4900.0	23.0	0.5	150.0
311	730.0	0.5	0.5	23.0
412	3.0	3.6	0.5	0.5
	220.0	3.6	0.5	0.5
614	61.0	0.5	0.5	23.0
715	1600.0	3.6	0.5	3.6
916	91.0	23.0	23.0	23.0
	59.0	23.0	23.0	0.5
217	54.0	0.5	0.5	93.0
	55.0	0.5	0.5	43.0
618	120.0	1100.0	3.6	0.5
	130.0	1100.0	3.6	0.5

Table B (continued)

SM	Rapid Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus (MPN/g)
Laboratory 13:				
201	540.0	210.0	3.0	0.5
	73.0	0.5	0.5	0.5
502	640.0	160.0	7.2	0.5
	82.0	7.2	0.5	0.5
403	30.0	0.5	0.5	0.5
	30.0	0.5	0.5	3.0
104	30.0	0.5	0.5	0.5
	30.0	0.5	0.5	0.5
305	31.0	43.0	0.5	9.1
	34.0	93.0	0.5	0.5
606	610.0	3.6	7.2	3.0
	760.0	3.6	0.5	0.5
707	20.0	0.5	0.5	93.0
	30.0	0.5	0.5	15.0
808	30.0	0.5	0.5	0.5
	30.0	0.5	0.5	0.5
309	30.0	23.0	0.5	3.6
311	1200.0	23.0	0.5	23.0
	1200.0	23.0	0.5	23.0
412	30.0	0.5	0.5	23.0
	30.0	9.1	3.6	3.0
513	30.0	0.5	0.5	0.5
	30.0	0.5	0.5	9.1
614	30.0	0.5	0.5	43.0
	30.0	0.5	0.5	23.0
715	48.0	9.1	0.5	9.1
	78.0	9.1	0.5	3.6
618	820.0	1100.0	0.5	0.5
	1900.0	1100.0	0.5	0.5
Laboratory 14:				
311	2000.0	0.5	0.5	240.0
	2400.0	0.5	0.5	240.0
412		23.0	0.5	43.0
		0.5	0.5	0.5

Table B (continued)

SM	Rapid Values			
	APC/g (x 1000)	Coliform (MPN/g)	E. coli (MPN/g)	Coagulase + Staphylococcus (MPN/g)
513		0.5	0.5	0.5
		0.5	0.5	23.0
614	1000.0	0.5	0.5	23.0
	37.0	0.5	0.5	23.0
715	410.0	0.5	0.5	23.0
Laboratory 15:				
808	3400.0	460.0	0.5	1100.0
309	100.0	15.0	0.5	9.1
412	30.0	0.5	3.6	0.5
	30.0	23.0	0.5	0.5
513	30.0	0.5	0.5	0.5
	30.0	0.5	0.5	0.5
715	230.0	0.5	3.0	3.0
916	300.0	1100.0	0.5	3.6
217	350.0	9.1	3.6	23.0
	400.0	3.6	9.1	15.0
618	210.0	1100.0	3.6	0.5
Laboratory 16:				
217	20.0	23.0	0.5	
	19.0	23.0	0.5	
618	190.0	1100.0	0.5	3.6
	188.0	1100.0	0.5	0.5

Table C. Composition of Plate Count Agar

Yeast Extract	3 g
Tryptone	5 g
Dextrose	1 g
Agar	15 g
Distilled water	1000 ml

Table D. Composition of Eugon Agar

Tryptose	15 g
Soytone	5 g
Dextrose	5 g
L-Cystine	0.2 g
Sodium chloride	4 g
Sodium sulfite	0.2 g
Sodium citrate	1 g
Agar	15 g
Distilled water	1000 ml

Table E. Composition of Lauryl Tryptose Broth

Tryptose	20 g
Lactose	5 g
Dipotassium phosphate	2.75 g
Monopotassium phosphate	2.75 g
Sodium chloride	5 g
Sodium Lauryl Sulfate	0.1 g
Distilled water	1000 ml

Table F. Composition of EC Medium

Tryptose	20 g
Lactose	5 g
Bile Salts No. 3	1.5 g
Dipotassium phosphate	4 g
Monopotassium phosphate	1.5 g
Sodium chloride	5 g
Distilled water	1000 ml

Table G. Composition of Brilliant Green Lactose Bile Broth (2%)

Peptone	10 g
Lactose	10 g
Oxgall	20 g
Brilliant green	0.0133 g
Distilled water	1000 ml

Table H. Composition of Levine EMB Agar

Peptone	10 g
Lactose	10 g
Dipotassium phosphate	2 g
Agar	15 g
Eosin Y	0.4 g
Methylene Blue	0.065 g
Distilled water	1000 ml

Table I. Composition of Trypticase Soy Broth

Tryptone	17 g
Soytone	3 g
Dipotassium phosphate	2.5 g
Dextrose	5 g
Sodium Chloride	100 g
Distilled water	1000 ml

Table J. Composition of Vogel-Johnson Agar

Tryptone	10 g
Yeast Extract	5 g
Mannitol	10 g
Dipotassium Phosphate	5 g
Lithium Chloride	5 g
Glycine	10 g
Agar	15 g
Phenol Red	0.25 g
Distilled water	1000 ml

Table K. Composition of Brain Heart Infusion Broth

Infusion from calf brains	250 g
Infusion from calf heart	250 g
Proteose peptone	10 g
Dextrose	2 g
Sodium Chloride	5 g
Disodium Phosphate	2.5 g
Distilled water	1000 ml

Table L. Composition of Tryptic Mannitol Meat Broth

Beef heart infusion	100 g
Tryptone	17 g
Soytone	3 g
Proteose peptone	4.5 g
d-Mannitol	2.5 g
Sodium Chloride	100 g
Dipotassium Phosphate	2.5 g
Phenol Red	0.12 g
Distilled water	1000 ml

Table M. Analysis of Variance for APC Observations

Source of Variation	d.f.	Mean Square	F Value Calculated	F Value d.f.
Total	333			
Source	9	122266.42	15.76**	9/284
Lab	15	11688.08	1.50	15/284
Technique	1	5273.60	0.68	1/284
Lab x technique	15	1144.16	0.14	15/284
Error	284	7753.41		

**p < 0.01

Table N. Analysis of Variance for Coliform Observations

Source of Variation	d.f.	Mean Square	F Value Calculated	F Value d.f.
Total	338			
Source	9	1192261.38	15.70**	9/289
Laboratory	15	149092.18	1.96*	15/289
Technique	1	24822.20	0.32	1/289
Lab x technique	15	16149.21	0.21	15/289
Error	289	75925.64		

**p < 0.01

*p < 0.05

Table O. Analysis of Variance for E. coli Observations

Source of Variation	d.f.	Mean Square	F Value Calculated	F Value d.f.
Total	338			
Source	9	544.88	6.70**	9/289
Laboratory	15	129.40	1.59	15/289
Technique	1	38.93	0.47	1/289
Lab x technique	15	58.13	0.71	15/289
Error	289	81.29		

**p < 0.01

Table P. Analysis of Variance for Coagulase-Positive Staphylococci Observations

Source of Variation	d.f.	Mean Square	F Value Calculated	F Value d.f.
Total	336			
Source	9	153490.14	4.53**	9/287
Laboratory	15	58926.92	1.74*	15/287
Technique	1	66487.68	1.96*	1/287
Lab x technique	15	46223.80	1.36	15/287
Error	336	33810.44		

*p < 0.05

**p < 0.01

VITA

The author was born on August 11, 1947, in Horse Cave, Kentucky. He attended John McDonogh High School in New Orleans, Louisiana, and was graduated in June 1966. He entered Southeastern Louisiana University and received his Bachelor of Science degree in December 1970.

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EXAMINATION AND THESIS REPORT

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Title of Thesis: Rapid Methods for the Bacteriological Analyses of Raw Frozen Breaded Shrimp

Approved:

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Major Professor and Chairman

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Date of Examination:

February 25, 1976